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THE RELATIVE RETENTION OF INFUSED CHLORIDE, UREA AND WATER

A. V. WOLF

From the Department of Physiology, University of Rochester School of Medicine, Rochester, N. Y.

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The following study was undertaken to demonstrate experimentally that the kinetics of excretion of both threshold and no-threshold substances possess fundamental similarities. In the unanesthetized dog a constant infusion of known composition was administered (intravenously in these experiments) for several hours, and all urine was collected, generally at one hour intervals, and analysed. Computations were made merely from the complete input and output information, blood analyses being generally unnecessary.

Definitions. The hypothesis is made that rate of excretion of any substance is proportional to the load of the substance in the body; and the animal is regarded as being initially in a state of balance with no appreciable loads (with a qualification for urea noted under Procedures), that is

$$uU = \gamma L = \gamma(A - A_T)b \tag{1}$$

where u is rate of urine flow in cc./min.; U is concentration of a urine solute in mgm./min.; L is its load in mgm.; A is its plasma concentration in mgm./cc.; A_T is its renal threshold concentration in mgm./cc.; b is its volume of distribution in cc.; and γ is a "velocity constant" of excretion of the substance. This equation is based on the law of exponential decay, and is applied to all substances regardless of threshold. It is useful, however, to note the significance of the velocity constant for no-threshold substances. By definition,

$$C = \frac{uU}{A} \tag{2}$$

where C is clearance.

By definition $$\gamma = \frac{uU}{L} = \frac{uU}{Ab} \tag{3}$$

Therefore, $$\gamma = \frac{C}{b} \tag{4}$$

Thus the velocity constant simply represents the ratio of the clearance of a no-threshold substance to its volume of distribution. Since γ is the constant of proportionality of rate of excretion to load, it also is to be found in the integral form

$$A_t = A_i e^{-\gamma t} \tag{5}$$

where A_t is the plasma concentration at time t, and A_i is the initial plasma level, in the case of a decaying load. Then

$$\gamma = \ln \left(\frac{A_i}{A_t} \right) \Big/ t \tag{6}$$

and the velocity constant is seen as the slope of the line relating ln A to time. From equations (6) and (4) it is clear that Barnett's (1940) k value in connection with inulin clearances is the velocity constant of inulin excretion, representing the ratio of the inulin clearance to its volume of distribution.

Dominguez (1934) originally showed some of the significance of the velocity constant of excretion. However, the experiments to be considered here include the velocity constant not as a useful end in itself, but as a means to an end, viz., the study of relative retention and excretion. Emphasis is placed upon it as a variable parameter rather than as a constant.

From an infusion of known rate and from the known excretion up to time t, we can compute L (load) and the velocity constant. No plasma analysis is required.

The theory of the isorrheic state. In 1940 Eggleton, Pappenheimer and Winton employed the term "isorrheic" to describe constant urine flow in the isolated kidney. The meaning of this useful term is here extended by examining the properties of the isorrheic state and of isorrheic infusions, *in vivo*.

If the constituents of a solution infused at a constant rate could appear in urine with the same relative concentrations of solute and solvent that they have in the infused fluid, we might expect, in time, a load of infused fluid to build up to a point so that the isorrheic state (isorrhea) would be established, i.e.,

$$uU = iI = \text{a constant} \tag{7}$$

where i is the rate of infusion in cc./min. and I is concentration of infused fluid in mgm./cc. If the velocity constant remained constant

$$\frac{dL}{dt} = iI - \gamma L \tag{8}$$

and on integration

$$L_t = \frac{iI(1 - e^{-\gamma t})}{\gamma} \tag{9}$$

and from equation (3),

$$(uU)_t = iI(1 - e^{-\gamma t}) \tag{10}$$

and when t becomes large, uU approaches iI.

For many substances there is a value (an upper limit) of U above which a steady state cannot be maintained. That is, the kidney can elaborate urine of

increasing concentration of substance x from infusions of increasing concentration of substance x until a point is reached at which further increase of I_x results in no marked increase of U_x; but to satisfy the urgency of removing the load of x, increase of u occurs. The highest urine concentration which can be maintained without relative retention of x to water (y) may be called the *limiting isorrheic concentration* (L.I.C.) and denoted by $[U]$. With a constant infusion of a concentration set so that $I_x = [U]_x$, a load of x can be reached so that output will equal input, that is, isorrhea will be established and

$$u[U]_x = iI_x \tag{11}$$

but since

$$[U]_x = I_x \tag{12}$$

$$u = i \tag{13}$$

Therefore as a result of the infusion of x whose concentration equals the L.I.C. of x, both solute and solvent reach isorrhea simultaneously. It is implicit here that the excretion rates of solute and of solvent (x and y, respectively) reach the same fraction of their infusion rates at time t. Thus

$$\frac{uU_x}{iI_x} = (1 - e^{-\gamma_x t}) = \frac{uU_y}{iI_y} = (1 - e^{-\gamma_y t}) \tag{14}$$[1]

Therefore

$$\gamma_x = \gamma_y \tag{15}$$

Hence an important property of a L.I.C. infusion is that the velocity constants of solute and solvent are equal; or the entire infusate may be regarded as possessing a characteristic velocity constant. At concentrations of I not equal to U, it will be shown that the velocity constants of solute and solvent may be neither equal nor constant.

In the work presented here the usefulness of the velocity constant is quantitatively examined. The velocity constant of excretion is a constant only under special conditions; but so long as these are understood and controllable, the velocity constant is a useful tool in the study of relative retentions of different substances, concentration ceilings and related problems.

PROCEDURES. The bladder trigones of female dogs were exteriorized so that urine could be collected accurately and continuously while each dog stood in a stall and was infused with various solutions by ear vein at a constant rate for approximately 7 hours. The usual rate of infusion was 2.3 cc. per minute. Dogs suffered no appreciable discomfort during the procedure.

Blood from leg veins was drawn into tubes containing potassium oxalate at the beginning, middle and end of an experiment, and the plasma was analysed for chloride or chloride and urea.

The average rate of excretion during a time period was related to the loads at the end of that period, no attempt being made at interpolation since such correction was of little consequence to the final analysis of the data. Load was computed as the difference between the amount of a substance infused and

[1] It is assumed that $uU_y = u$ and $iI_y = i$.

the amount excreted by the end of a period. The average rate of excretion during this period divided by the load was called the velocity constant. The load of solute divided by the load of solvent (water) was called the *retention concentration* $\left(\frac{L_{\text{solute}}}{L_{\text{water}}}\right)$, and was a measure of the virtual concentration of the volume of infused fluid retained at the end of a time period, uncorrected for insensible loss of water. The ratio of the retention concentration to the infused concentration was called the *relative retention* $\left(\frac{L_{\text{solute}}}{L_{\text{water}} \cdot I}\right)$. So long as we neglect insensible water loss, if the relative retention were maintained at unity, the solute and solvent would be excreted at equal rates per unit load, i.e., $\gamma_{\text{solute}} = \gamma_{\text{water}}$. If the relative retention were maintained greater than unity, solute would accumulate relative to water ($\gamma_{\text{solute}} < \gamma_{\text{water}}$); and if the relative retention were maintained less than unity, water would accumulate relative to solute ($\gamma_{\text{solute}} > \gamma_{\text{water}}$).

Insensible water loss was estimated during the experiment from weighings of the dog and stall on a balance adapted to the purpose. The difference between fluid weight infused and sensible weight removed (urine and blood samples) differed from the balance-measured weight change in any time period by the insensible loss.

Animals were kept on no special diet. Daily variations in their salt and urea content apparently made little difference to the consequences of presenting them with a 7 hour load of infusate. Thus an animal was always regarded as having a zero salt and water load at the beginning of an experiment. In the case of urea, a no-threshold substance, the computation of the velocity constant was modified by adding to the infused load approximately 2000 mgm./17 kgm. body weight/20 mgm. per cent plasma level (pre-experimental) of urea. This inclusion of the normal body content of urea in the load of that substance is necessary if the velocity constant of urea is to have the same kinetic significance as the velocity constants of chloride and water.

Chloride concentrations are reported as milligrams of sodium chloride per cubic centimeter, although no implications concerning the behavior of sodium are intended. All the data presented below unless otherwise specified refer to the state of the animal at the end of a 7 hour infusion.

Results. Retention concentrations of chloride change with the duration of infusion (fig. 1). The most significant fact, perhaps, is the tendency of all curves below 17 mgm./cc. at zero time to approach the 6 mgm./cc. retention concentration asymptotically. And all curves above 17 mgm./cc. proceed to higher values without limit. That 6 mgm./cc. is an asymptote will be shown presently. Commonly the initial trend of the retention concentration (when below 17 mgm./cc.) is the opposite to that displayed later, but this offers little hindrance to attaining the 6 mgm./cc. value because relatively small volumes of urine are excreted initially (compared to later ones) and the infused loads in early periods are likewise relatively small.

Table 1 is a protocol indicating in detail how excretion rates, velocity constants, retention concentrations, etc., vary with the duration of infusion.

Figures 2 and 4 relate the velocity constants of water and chloride, and water and urea, respectively, to infusion concentrations (urea infusions contained NaCl in concentration of either 6 or 17 mgm./cc., approximately). Despite the gross variability of the data plotted, the velocity constant of water tends to go through a minimum (fig. 2) and the velocity constant of chloride rises linearly. The higher value of I (17 mgm./cc.) at which the velocity constants of water and of chloride are equal is the *limiting isorrheic concentration* (L.I.C.). The lower value of I (6 mgm./cc.) at which these velocity constants are equal may be called the *non-limiting isorrheic concentration* (NL.I.C.).

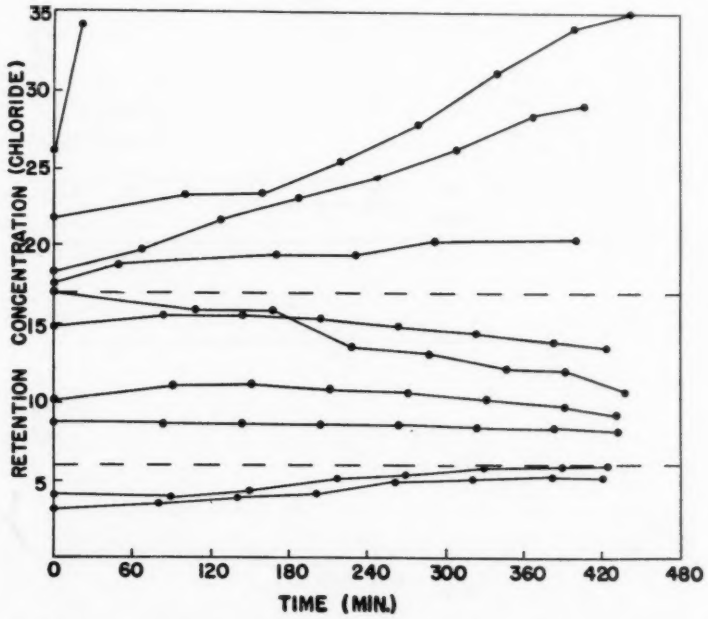

Fig. 1. Relative retention in relation to time of infusion. The experiments illustrate the tendency of an infusion of chloride of concentration less than 17 mgm./cc. (upper horizontal broken line) to be retained in time as 6 mgm./cc. (lower horizontal broken line). Infusion concentrations greater than 17 mgm./cc. are not tolerated indefinitely.

The velocity constant of urea (fig. 4) is rather constant after an initial "augmentation" concomitant with the increased rate of urine flow at 7 hours at high I_{urea} values as compared with low I_{urea} values. A L.I.C. of urea is evident at about $I = 35$ mgm./cc. There is no NL.I.C.

The urine concentrations also change with I (figs. 3 and 5) to bring about these relations. When I_{Cl} is less than 6 mgm./cc., U_{Cl} is always less than I_{Cl}, and so the velocity constant of chloride is less than that of water. When I_{Cl} is greater than 6 and less than 17 mgm./cc. (neglecting insensible loss for the present), U_{Cl} is greater than I_{Cl}. Thus the velocity constant of chloride is greater than that of water. When I_{Cl} is greater than 17 mgm./cc. the urinary

chloride concentration does not increase appreciably under the conditions of these experiments and the velocity constant of chloride is less than that of water, so that the relative retention of chloride increases with increasing duration of infusion. U_{urea} is always greater than I_{urea} below the L.I.C. so that the

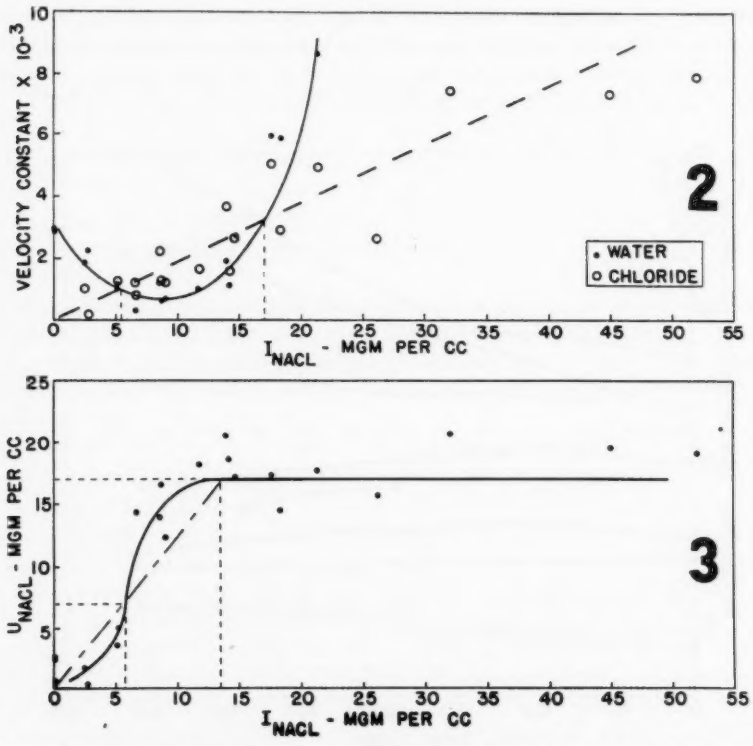

Fig. 2. Values found for velocity constants in relation to infusion concentration after 7 hours of infusion at 2.3 cc./min. The points of intersection of the curves represent the non-limiting isorrheic concentration and the limiting isorrheic concentration. Dogs 5 and 6. Wts. 18 and 10 kgm., respectively.

Fig. 3. Urinary concentration of chloride in relation to infused concentration of chloride. The broken line rising from the origin has a slope of 1.2 which corrects for insensible water loss. Dogs 5 and 6.

velocity constant of urea is greater than that of water below this value of 35 mgm./cc.

The relative retention of chloride as a function of I_{Cl} at the end of 7 hours is presented in figure 6. Curve $AA'A''$ based on the experiments of figure 2 indicates that at infusion concentrations of less than 6 mgm./cc., chloride is retained relative to water. This is true also above 17 mgm./cc. Between these critical points (NL.I.C. and L.I.C.) water is retained relative to chloride.

From figures 2 and 6 it follows that if the relative retention were maintained at unity, the velocity constants of water and chloride would be equal.

A similar relation holds for urea (fig. 8) although having only one critical point (a L.I.C.) the relative retention of urea is unity at one point only. This graph indicates that the L.I.C. is about 27 mgm./cc. The plot of retention

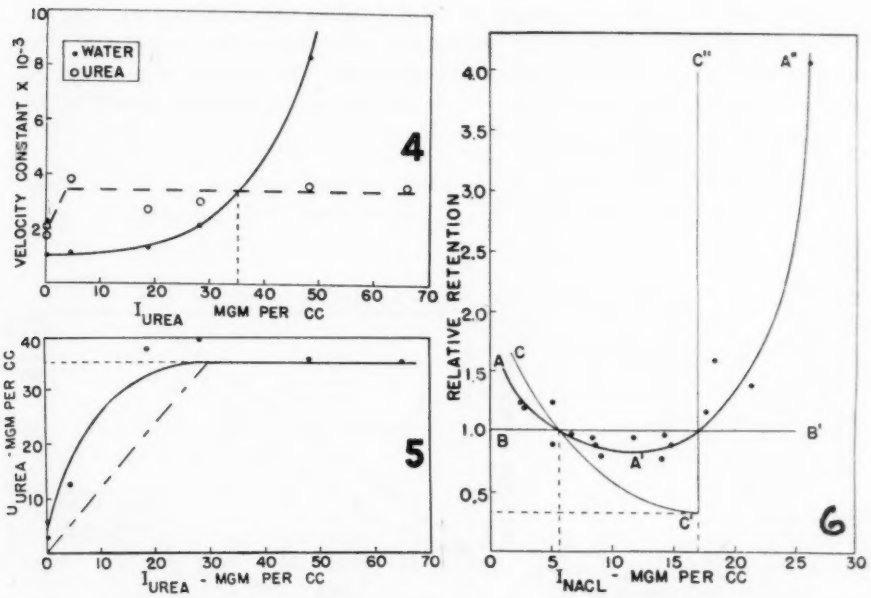

Fig. 4. Values found for velocity constants in relation to infusion concentration after 7 hours of infusion at 2.3 cc./min. Infusion contained 6 mgm. of chloride per cubic centimeter. There is only one point of intersection, i.e., only a limiting isorrheic concentration. Dog 5.

Fig. 5. Urinary concentration of urea in relation to infused concentration of urea. The broken line rising from the origin has a slope of 1.2 which corrects for insensible water loss. Dog. 5.

Fig. 6. Relative retention of chloride as a function of I_{Cl}. Curve $AA'A''$ is for the 7th hour determinations. Curve BB' is for zero time. Curve $CC'C''$ is for infinite time. All intersect mutually at critical points, i.e., at the non-limiting isorrheic concentration and limiting isorrheic concentrations of chloride. Dogs 5 and 6.

concentration against I_{urea}, also in figure 8, indicates 30 mgm./cc. as the L.I.C. (where infusion and retention concentrations are equal). The value of 35 mgm./cc. for the L.I.C. of urea is probably a better one as is suggested by figures 4 and 5.

Figure 7 illustrates a useful relation from which the L.I.C. and NL.I.C. may be drawn. By combining pairs of velocity constants of water and chloride as a ratio in a single experiment, critical values of 6 and 17 mgm./cc. may be estab-

lished for those retention concentrations at which the ratio of the velocity constants is unity.

All the seventh hour findings on chloride and water not represented in the various graphs are collected in table 2. Corresponding figures on urea and water (and chloride) are presented in table 3. These tables extend the observations to other dogs, and support the inferences drawn from the graphs.

It was noted after several hours of infusion of chloride of about 80 mgm./cc. concentration, that signs of salt intoxication (Guttman, 1865) were evident in tremor of the legs and head, increased restlessness and a thirst, presumably exquisite, judging from the fact that the act of turning on the water faucet within

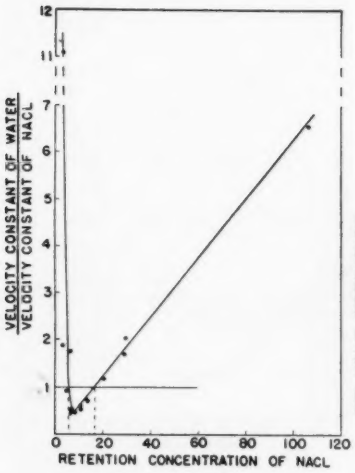

Fig. 7

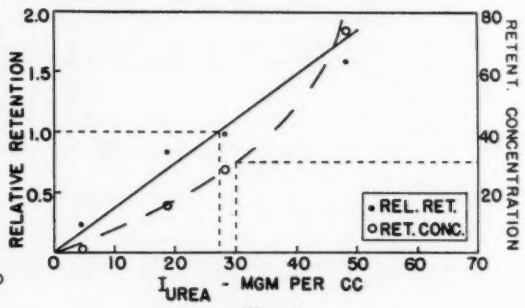

Fig. 8

Fig. 7. Non-limiting and limiting isorrheic concentrations are those retention concentrations of Cl at which the velocity constants of chloride and water are equal (i.e., when their ratio is unity). Dogs 5 and 6.

Fig. 8. Retention concentration and relative retention of urea as a function of infusion concentration of urea. Dog 5.

sight of the dog was sufficient to excite it violently. Such infusions were stopped before 7 hours for fear of killing the dogs. The velocity constants of chloride and water began to fall rapidly in the later, violent stages, perhaps indicating the onset of serious injury. Dogs having salt intoxication will, if they retain sufficient motor control, drink water (or their urine) greedily until they are forced to vomit. Then they drink further. The solution to their problem seems to be to excrete the excess salt in due time rather than to dilute it all at once, the latter process probably calling for many liters of water. The dogs recovered from this treatment with no evidence of permanent injury.

The results indicate that the dog seeks to retain infused chloride at a virtual concentration of 6 mgm./cc., excreting relatively more chloride than water from more concentrated infusates, and excreting more water than chloride from less

concentrated infusates. The dog cannot successfully continue to eliminate chloride from infusions more concentrated than 17 mgm./cc. of chloride, or to eliminate urea from infusions more concentrated than 35 mgm./cc. of urea. Dehydration (negative water loads) and relative retention of solute to water set in, increasing with the duration of such concentrated infusions.

Urea excretion obeys the law of exponential decay rather exactly over wide ranges of its concentration in infusates. Chloride and water excretion do not simply follow this law of decay except in cases of infusion with I_{Cl} set at 6 or 17 mgm./cc.; but they vary with the infusion concentration of chloride as well as with their respective loads.

Insensible water loss. In these experiments where the rate of infusion was generally about 2.3 cc./min., the factor of insensible loss was found to be appreciable. For dog 5, this averaged 0.37 cc./min. and for dog 10, about 0.43 cc./min., or approximately 16 per cent and 19 per cent of the infusion rate, respec-

TABLE 1

A protocol of experiment 64 (table 2)

I_{Cl} = 3.1 mgm./cc.

TIME	INFUSED CHLORIDE	EXCRETED CHLORIDE	LOAD OF CHLORIDE	EXCRETION RATE OF CHLORIDE	VELOCITY CONSTANT OF CHLORIDE	I_{Cl}/I_{H_2O}	RELATIVE RETENT. OF CHLORIDE	PLASMA CONC.	INFUSED WATER	EXCRETED WATER	LOAD OF WATER	EXCRETION RATE OF WATER	VELOCITY CONSTANT OF WATER
min.	*mgm.*	*mgm.*	*mgm.*	*mgm./min.*	*min.*$^{-1}$	*mgm./cc.*		*mgm./cc.*	*cc.*	*cc.*	*cc.*	*cc./min.*	*min.*$^{-1}$
88	655	142	513	1.61	0.00314	2.88	0.93		211	33	178	0.376	0.00211
148	1100	296	804	2.56	0.00319	2.98	0.96	7.0	356	86	270	0.884	0.00327
208	1550	460	1090	2.78	0.00256	3.09	1.00		500	148	352	1.03	0.00294
268	1990	610	1380	2.42	0.00175	3.08	0.99		644	194	450	0.767	0.00170
328	2440	730	1710	2.09	0.00122	3.24	1.04		787	260	527	1.10	0.00209
388	2890	920	1970	3.16	0.00160	3.43	1.10		931	355	576	1.58	0.00274
428	3180	1070	2110	3.94	0.00187	3.57	1.15	6.4	1030	440	590	2.07	0.00350

tively. Thus the L.I.C. of urine may not be so low as the L.I.C. of infusion fluid and U and I may never be maintained equal to one another. The broken line rising from the origin in figures 3 and 5 has a slope of $i/(i - w)$ where w was taken as about 20 per cent of i. Above and below this line U is to be found at the end of an infusion of finite duration. Theoretically as the relative retention approaches unity with increased time (when I is less than 17 mgm./cc.), all points will lie on such a line.

Discussion. *The velocity constant as a function of infusion concentration.* The relatively constant urea velocity constant which is found above an apparent "augmentation" limit (fig. 4) regardless of I_{urea} or I_{Cl}, is a measure of the fact that the law of decay accurately describes the excretion rate of this substance. However, the linear rise of the chloride velocity constant with I_{Cl} (fig. 2) gives no such description. Since

$$\gamma_{Cl} = KI_{Cl} \tag{16}$$

where K is a constant, then from (3)

$$uU_{Cl} = KL_{Cl}I_{Cl} \tag{17}$$

Equation (17) states that rate of chloride excretion is proportional not only to load but also to infusion concentration. It might be held that the law of

TABLE 2

Seventh hour data from infusions of chloride without urea

Dog weights: 10, 15 kgm.; 11, 16 kgm. Data not appearing in the graphs are to be found in this table.

DOG NO.	TIME	SUB-STANCES	I	TOTAL INPUT	LOAD	EXCRE-TION RATE	VELOCITY CONSTANT	L_{Cl}/L_{H_2O}	RELATIVE RETEN-TION	H_2O/Cl VELOCITY CON-STANT RATIO	PLASMA CONC.
	min.		*mgm./cc.*	*mgm. or cc.*	*mgm. or cc.*	*mgm. or cc./min.*	*min.*$^{-1}$	*mgm./cc.*			*mgm./cc.*
10	425	NaCl	0.0	0.0	0.0	0.76					5.8
		H_2O		994	530	2.55	0.00481				
10	428	NaCl	3.1	3180	2110	3.94	0.00187	3.57	1.15	1.87	6.4
		H_2O		1030	591	2.07	0.00350				
11	416	NaCl	3.1	2980	2700	1.53	0.00057	3.51	1.13	3.62	6.9
		H_2O		963	767	1.53	0.00200				
10	424	NaCl	3.5	3480	1470	4.94	0.00336	3.28	0.94	1.21	6.9
		H_2O		992	449	1.83	0.00408				
10	425	NaCl	4.0	3800	3440	1.25	0.00036	5.93	1.48	5.40	6.5
		H_2O		983	582	1.14	0.00196				
10	428	NaCl	7.0	7070	4910	12.0	0.00244	7.30	1.04	1.26	6.4
		H_2O		1010	673	2.07	0.00308				
10	432	NaCl	10.5	10600	6840	19.4	0.00284	9.35	0.89	0.57	6.9
		H_2O		1010	731	1.18	0.00162				
10	424	NaCl	14.8	14500	7100	39.3	0.00554	13.5	0.92	0.79	7.3
		H_2O		980	525	2.30	0.00437				
10	438	NaCl	17.1	17400	3820	39.4	0.0103	11.7	0.68	0.62	8.4
		H_2O		1020	326	2.06	0.00638				
11	440	NaCl	21.7	22600	8260	41.2	0.00499	35.1	1.62	1.94	7.3
		H_2O		1040	235	2.27	0.00965				
10	420	NaCl	24.8	24800	10400	60.0	0.00580	46.9	1.89	2.43	7.8
		H_2O		1000	221	3.11	0.0141				
10	429	NaCl	36.6	36200	7190	96.0	0.0134	−15.4	−0.42	−0.77	8.4
		H_2O		991	−467	4.80	−0.0103				
11	408	NaCl	49.5	46900	10500	110.0	0.0104	−12.0	−0.24	−1.79	9.0
		H_2O		946	−877	5.14	−0.00585				

decay actually applies here as with urea, but that another factor, I, modifies the expression of the law. What would be the influence of I_{Cl} on the chloride velocity constant? Why should the rate of excretion of chloride at $I = 17$ mgm./cc. be three times greater than when $I = 6$ mgm./cc., for a given load of chloride?

TABLE 3

Seventh hour data from infusions of urea (and chloride)

DOG NO.	TIME	SUBSTANCES	*I*	INPUT	LOAD*	EXCRETION RATE	VELOCITY CONSTANT	L_{solute}/L_{H_2O}	RELATIVE RETENTION	H_2O/Cl VELOCITY CONSTANT RATIO	H_2O/UREA VELOCITY CONSTANT RATIO	PLASMA CONC.	INCREMENT OF PLASMA CONC.
	min.		*mgm./cc.*	*mgm. or cc.*	*mgm. or cc.*	*mgm. or cc./min.*	*min.*$^{-1}$	*mgm./cc.*				*mgm./cc.*	*mgm./cc.*
5	422	NaCl	2.8	3080	2320	0.47	0.00020	3.32	1.19			7.8	
		H_2O		1060	699	1.55	0.00222			11.1	10.5		
		Urea	0.0	0	2000	4.21	0.00211	0				0.204	−0.016
5	415	NaCl	5.1	5050	3450	4.16	0.00121	4.50	0.88			6.3	
		H_2O		989	768	0.84	0.00109			0.902	0.613		
		Urea	0.0	0	2800	4.98	0.00178	0				0.208	−0.120
5	423	NaCl	5.9	5640	3880	8.15	0.00211	5.16	0.88			5.9	
		H_2O		953	750	0.87	0.00115			0.545	0.300		
		Urea	4.7	4400	2840	10.9	0.00384	1.11	0.24			0.36	0.14
10	409	NaCl	6.0	5190	1610	8.55	0.00533	2.36	0.394			7.9	
		H_2O		868	680	0.60	0.00089			0.166	0.100		
		Urea	9.06	7850	2740	24.1	0.00882	−0.39	−0.043			0.54	0.12
5	410	NaCl	5.8	5370	3090	8.15	0.00264	4.43	0.76			6.1	
		H_2O		925	697	0.96	0.00137			0.520	0.492		
		Urea	18.8	17300	13000	36.2	0.00279	15.8	0.837			0.84	0.61
5	425	NaCl	6.0	5750	2590	9.27	0.00357	4.43	0.74			7.0	
		H_2O		956	586	1.27	0.00217			0.607	0.710		
		Urea	28.1	26900	16600	50.8	0.00306	27.8	0.990			1.18	0.93
10	428	NaCl	6.4	5750	1610	14.5	0.00900	4.43	0.691			6.2	
		H_2O		900	365	2.45	0.00672			0.747	0.990		
		Urea	33.8	30400	11300	76.5	0.00680	17.1	0.507			1.30	0.79
5	407	NaCl	4.6	4120	1030	11.8	0.0115	3.44	0.748			7.1	
		H_2O		917	299	2.50	0.00837			0.729	2.32		
		Urea	48.1	44000	24700	89.4	0.00361	75.7	1.57			1.84	1.53
5	402	NaCl	7.0	6250	1680	16.2	0.00963	−120	−17.2			8.8	
		H_2O		854	−14	3.23	−0.231			−24.0	−64.3		
		Urea	65.9	56100	31700	114	0.00360	−2120	−32.2			2.64	2.45
10	417	NaCl	6.4	6550	−1010	25.3	−0.0252	3.69	0.58			7.6	
		H_2O		1021	−273	4.44	−0.0162			0.643	−0.284		
		Urea	75.5	77300	30700	175	0.00570	−112	−1.49			3.45	3.12
10	422	NaCl	17.0	17500	5700	30.8	0.00541	11.5	0.675			8.1	
		H_2O		1030	496	1.45	0.00292			0.540	0.707		
		Urea	9.18	9460	4530	18.7	0.00413	2.07	0.226			0.470	0.06
10	414	NaCl	17.6	17000	5450	38.6	0.00708	13.0	0.740			8.5	
		H_2O		994	419	1.97	0.00471			0.666	1.13		
		Urea	19.1	19000	9790	40.8	0.00417	17.4	0.910			0.833	0.530
10	429	NaCl	16.7	17200	3250	42.5	0.0131	−37.4	−2.24			8.0	
		H_2O		1030	−87	3.31	−0.0381			−2.91	5.57		
		Urea	51.6	53100	18200	124	0.00684	−179	−34.6			2.13	1.81

* In case of urea, the value given for "load" actually represents the load plus the normal body content.

Distortion. It seems feasible, for descriptive purposes, to interpret the increase of the chloride velocity constant with increasing I_{Cl}, when no other solute is in the infusion fluid, independently of direct consideration of glomerular filtration rate.

Consider the volume of distribution of water in the body divided by a semipermeable membrane into a chloride and a non-chloride space, b_{Cl} and b_d, respectively. Assuming the system a perfect osmometer, regard the concentration of chloride in b_{Cl} as A_{Cl}. At equilibrium, the effective concentration in b_d may also be called A_{Cl}. It can be shown from such considerations that upon the addition of a load of chloride solution to the chloride space

$$\frac{I_{Cl}}{A_{Cl}} = f\left[\frac{\left(\frac{b'_{Cl}\, b_d}{b'_d}\right) - b_{Cl}}{L_{water}}\right] \tag{18}$$

where b'_{Cl} is the new chloride space, b'_d is the new non-chloride space, and f represents "a function of."

This expression indicates that the ratio I/A is a measure of the departure from normal of the relative sizes of chloride and non-chloride space per unit volume of infusate. Whether the animal acts as a perfect osmometer is not an important question here. Within limits it seems like such a system (Lands, Cutting and Larson, 1940). This ratio, for some purposes may be called the *distortion* of the chloride space, and as a number, indicates by its magnitude the degree of imbalance of the normal relations of volume of the chloride to the non-chloride space. The product of the distortion and L_{water} represents some function of the *distorted volume*. The discrepancy between such a theoretical distorted volume and the real erratic volume is unimportant so long as some type of volume shift is induced by chloride infusion. An infusate containing 6 mgm. chloride per cubic centimeter induces a distortion of nearly 1. A pure water infusion would induce no distortion of chloride space.

A tenable hypothesis seems to be that although the excretion rate of a substance is ordinarily proportional to load, the kidney might be obligated not only to discharge loads the more rapidly as they are the greater, but also to maintain the normal ratio of the solute and non-solute volumes of distribution. This concept has been considered by Peters (1942). In the case of chloride excretion, the distortion factor may be regarded as at least equal in influence to concentration or load factors. Urea should show no distorting influence since its volume of distribution is common with that of water. Without distortion, the law of exponential decay is revealed for urea in a relatively uncomplicated fashion. The unknown way in which a distortion factor operates, if it has significance at all, may make suspect its case for recognition as an entity. How a distorted volume could influence the nephrons of the kidney is unknown unless it be through changes in the kidney's own intraspatial relations.

It is believed that the excretion rate of chloride, when it is infused with no other solute, may be related to two factors: 1, load, and 2, distortion. The latter is an expression of the departure from normal of the relative volumes of chloride and non-chloride spaces and is a function of the ratio I/A. An increase

of distortion (increase of I/A) is concomitant with an increase in the excretion rate of chloride per unit load of chloride. If excretion rate were simply proportional to super-threshold concentration, then infusions of $I = A_T$ might never be excreted since the super-threshold concentration $(A - A_T) = O$. This supports the contention that a load can be excreted at a rate related to the distorted volume which it creates. In contrast, the excretion rate of urea seems to depend only on load (above an augmentation limit). Since urea and water occupy approximately the same volume of distribution, the urea space is not distorted appreciably. This lack of a distortion factor for urea is in harmony with the nicety with which urea excretion apparently obeys the law of exponential decay.

The velocity constant as a function of infusion duration. The kidney apparently operates to convert the volume of infused fluid to a retained concentration of 6 mgm./cc. Thus we may view the NL.I.C. of 6 mgm./cc. as a sort of "physiological saline" elected by the animal and guarded by the kidney.

One of the most important factors in determining the shape of the curve $AA'A''$ (fig. 6) is the duration of the infusion. Thus at zero time after infusion, the retention concentration must be equal to the infusion concentration since no time has elasped for the transformation of the infusate by excretion. And the curve BB' is the theoretical curve obtained at zero time; the relative retention is unity independently of I_{Cl}. If an infusion proceeded for infinite time, an infusion of concentration just short of 17 mgm./cc. would have been converted to a retention concentration of 6 mgm./cc. and the relative retention would be 6/17 or 0.35. This would be the lowest possible relative retention under these conditions. If the infusion concentration exceeds 17 mgm./cc. then at infinite time, the retention concentration would become infinite and the relative retention would be infinite. Any I value less than 17 mgm./cc. should in infinite time give rise to a relative retention of $6/I$ and thus the curve $CC'C''$ is derived. It may be observed how the 7 hour curve $AA'A''$ fits between the zero time and infinite time curves.

Thresholds. Attention is called to the concept of thresholds by the finding of the tendency to retain a "physiological saline." Rehberg (1926) suggested that thresholds ought to be defined only for actively resorbed substances. Using a reference substance (creatinine) at that time presumed to be neither secreted nor resorbed by tubules, he found that when chloride fell below about 6.2 mgm./cc. in the plasma, active resorption occurred (since the concentration of chloride in the resorbed fluid was higher than the plasma concentration), and above 6.2 mgm./cc. no active resorption was apparent and chloride acted like a no-threshold substance. This is sensibly the same conclusion arrived at in the present work, although only chloride analyses of urine and infusion fluid are required by the technique. The present work supports the rationale of the Rehberg threshold, further defining it as a NL.I.C. of chloride, or a plasma concentration above which chloride is excreted relative to water and below which chloride is retained relative to water.

The difference between the maximum and limiting isorrheic concentrations of urine. That U can ever exceed its L.I.C. brings up the question as to why the L.I.C.

should be different from the maximum urine concentration. A partial answer to this may be ventured, although it is probable that a great deal of evidence remains to be gathered on this matter. This answer concerns the notion that if the infusion of urea which would ordinarily bring about a given U_{urea} value, had contained sufficient chloride to induce a greater urine flow at the end of some period, the U_{urea} would be expected to have a smaller value so that the rate of excretion of urea for the same load of urea would be a constant. The rise of U on a falling urine flow is also consonant with maintaining a constant urea velocity constant although the reasons for the rapid decline of the velocity constant of water under these conditions is not fully clear. Urine containing as much as 25 mgm./cc. chloride has been found, a deviation from the L.I.C. of 47 per cent.

SUMMARY

1. A technique for studying the relative retentions of constantly infused solutes and water is described, and theoretical aspects of the isorrheic state (constant urine flow or solute excretion with constant infusion rate) are developed.

2. The velocity constants of excretion (rate of excretion per unit load) of chloride, water, and urea have been ascertained in the dog. They may be used to characterize those critical concentrations of infusion fluid and urine at which neither solute nor water are retained from an infusion, relative to one another. For chloride, expressed as sodium chloride, these concentrations are approximately 6 and 17 mgm./cc. For urea the only such concentration is approximately 35 mgm./cc. (when the simultaneously infused chloride is less than 17 mgm./cc.) under the conditions of these experiments.

3. The law of exponential decay is regarded as the underlying pattern followed by the excretion of threshold as well as no-threshold substances, and factors which relate to the modification of this pattern are examined. Excretion rate of chloride can be expressed not only as a function of load but also as a function of the distortion, or departure from normal, of the ratio of chloride to non-chloride space, induced by this load. The excretion of urea is uncomplicated by a distortion factor.

4. Rehberg's concept of threshold concentration for ions like chloride is extended and a simple technique for determining and expressing such threshold concentrations is suggested.

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THE BEHAVIOR OF THE SPLEEN IN HEMORRHAGIC HYPOTENSION AND SHOCK[1]

R. N. LEWIS, J. M. WERLE AND C. J. WIGGERS

From the Department of Physiology, Western Reserve University Medical School, Cleveland, O.

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In cases of death from shock, the spleen at autopsy has been reported as small, firm, dry, contracted or anemic, as well as large, soft and congested. In experimental shock following intestinal exposure, Y. Henderson (1) noted distention of the spleen. Erlanger and Gasser (2) describe the spleen as "often enlarged and containing hemorrhagic areas" after caval occlusion, aortic clamping and intestinal exposure. Whipple, Stone and Bernheim (3) mention splenic congestion in some of their dogs that died from intestinal obstruction. Moon (4), on the contrary, insists that the organ contains less blood than normally and in the dog is dry, firm and contracted. Lindgren (5) who used the Sjödstrand method for evaluating blood volume of organs by staining red corpuscles only, reported reduction in blood content of the spleen in guinea pigs that died from histamine or operative shock. However, they used spleens of decapitated animals (hemorrhage!) as a criterion of the normal. Zwemer and Scudder (6) described a red and leathery spleen in dogs that died from traumatic shock. Moore (7) who determined the spleen/body weight ratios of cats, shocked in various ways, found that the ratios agreed with the smallest values previously reported by Barcroft for normal cats. In reviewing the autopsy protocols of 48 experiments reported upon by Werle, Cosby and Wiggers (8), we find that the spleen was described as small, hard or maximally contracted in thirty-nine, normal or slightly contracted in six, and large or engorged in three experiments. However, the difficulty of deciding how large a spleen ought to have been if the animal had died a natural death often made decisions difficult in the instances in which it was classed as "normal."

Since the spleen shrinks at death, regardless of the manner in which this occurs (Barcroft, 9), observations of spleen size made post mortem cannot be expected to yield much information with regard to its size during life. For these reasons, we studied the changes in the size of exteriorized spleens during the course of hemorrhagic hypotension and hemorrhagic shock by the method of Barcroft and Stephens (10).

METHODS. Dogs were anesthetized with morphine and sodium barbital as in previous studies (8). Subclavian pressures were recorded optically by calibrated optical manometers of the Gregg type and by turning a 3-way cock mean pressure could also be read on the calibrating manometer. A femoral artery was cannulated for bleeding and a femoral vein for reinjecting the blood.

The spleen was exteriorized through a small midline incision and laid on a flat surface, taking care not to exert tension or torsion on its pedicle. It was loosely

[1] This investigation was supported by a grant from the Commonwealth Fund.

covered with cellophane to prevent drying. Its contour was traced with a wax pencil on a glass plate held in juxtaposition to the spleen and the outline so obtained was transferred to paper. The area was measured with a planimeter. Frequent duplicate outlines and measurements were made as checks. Since, for our purpose, nothing was gained by translating surface areas into probable volumes as described by Barcroft and Stephens (10), our results are reported in terms of area and the changes as percentage variations from the controls before bleeding. These were obtained after completion of operative procedures and allowance of a 20 to 30 minute interval for attainment of circulatory equilibrium.

When control sizes remained uniform, the dogs were bled at a moderate rate (ca. 100 cc./6min.) until mean arterial pressure was reduced approximately to 50 mm. Hg. The animal was allowed to recover for ½ to 1 hour and a second slower hemorrhage continued until the pressures were reduced to 40 mm. or below and held there for 45 minutes to 2 hours, the exact time depending on the animal's resistance and the object of the experiment. When necessary, small quantities of blood were reinfused to maintain viability of the respiratory center. After the period of hypotension, the total volume of withdrawn blood—prevented from clotting by addition of liquaemin[2] (Roche) and properly strained—was reinjected, as in experiments previously reported (8). The subsequent course of events depended on the intensity and duration of the hemorrhagic hypotension.

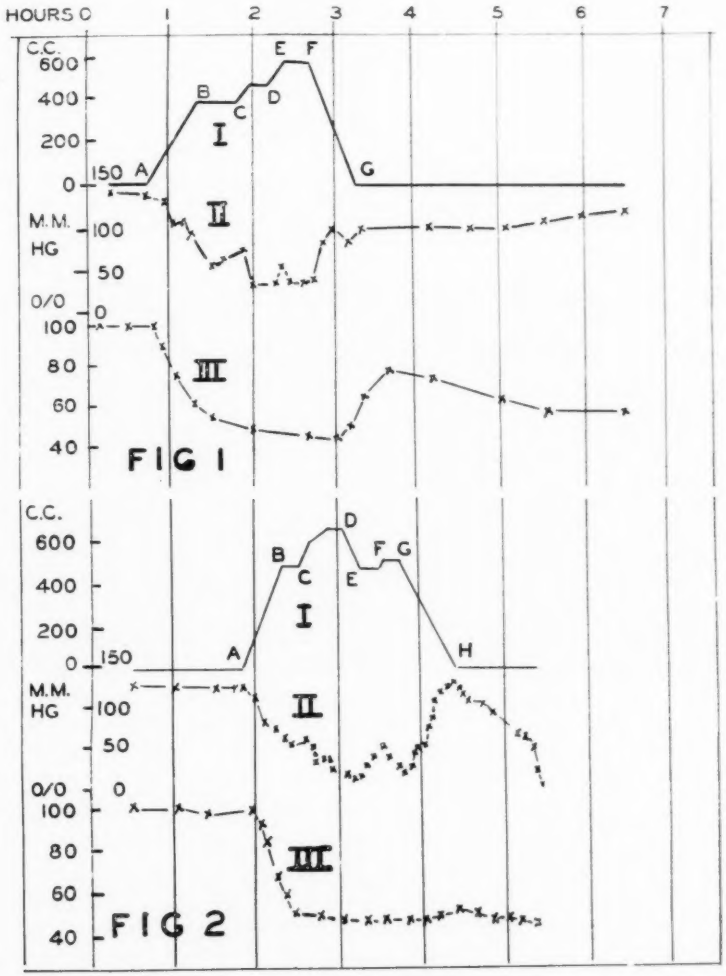
Results. It should be stated that, in a control experiment on a dog under similar morphine-sodium barbital anesthesia, the size of the exteriorized spleen remained practically constant for a period of six hours, and during the ensuing 2½ hours gradually decreased to 90 per cent of these values.

In order to study the effects of a brief period of extreme hypotension, two experiments were performed in which this lasted 45 minutes only. Then all the blood was reinjected. Results from one of these are shown in figure 1. After withdrawal of 366 cc. at *A-B*, mean arterial pressure was reduced to 54 mm. and the optical pulses showed all the characteristics of a severe hemorrhage, previously described (8). The spleen size decreased to 57 per cent of the control and continued to decrease to 49 per cent during the compensatory rise of pressure to *C*. At this time, the animal was bled an additional 90 cc. and later, *D*, an additional 100 cc. This maintained arterial pressures approximately at 30 mm. for 45 minutes. The spleen contracted further to 43 per cent of its control size. It should be noted that the greatest decrease occurred during the initial hemorrhage which confirms results of Barcroft et al. (10) and Grindlay et al. (16).

With reinfusion of all the withdrawn blood *F-G*, mean arterial pressures recovered to 100 mm. Hg. For 3½ hours thereafter arterial pressure pulses of good form were maintained and the mean pressure gradually rose. The spleen increased to 78 per cent of its normal size during reinfusion, but thereafter, and without significant changes in arterial mean pressures or pressure pulses, it gradually decreased again to 55 per cent of the control. The animal was sacri-

[2] We are indebted to Roche-Organon, Inc., Nutley, New Jersey, for their generous supply of the liquaemin solution used in these experiments.

ficed at the end of 6½ hours and at autopsy revealed no gross pathological changes, including the duodenal mucosa.



Figs. 1–2. Curve *I*, volume of blood withdrawn upward, reinfusion downward. Curve *II*, changes in mean arterial pressure. Curve *III*, percentage changes in area of exteriorized spleen.

Figure 2 shows results on an 18 kgm. dog. The mean blood pressure at the beginning of the experiment was 125 mm. Hg. After a control period of 90 minutes *A*, blood was withdrawn from the animal at a fairly uniform rate of 100 cc. per six minutes until the blood pressure had dropped to 55 mm. Hg, *B*.

The total amount of exsanguinated blood at this time was 500 cc. The animal was allowed to equilibrate at this pressure for a period of fifteen minutes. The respirations during this period were increased in both rate and depth. At *C-D* an additional 150 cc. of blood was removed and the mean blood pressure fell to 20 mm. Hg. The spleen area decreased progressively during the initial hemorrhage, but not much more during the second bleeding. When the mean blood pressure reached 20 mm. Hg the respirations were very shallow, heart action was feeble and it was thought advisable to reinject 200 cc. of blood in order to save the animal, *D-E*. As a result of this injection, the mean blood pressure gradually rose to 50 mm. Hg, but the spleen area did not show a corresponding increase. In order to maintain a pressure level less than 50 mm. Hg, the dog was bled another 25 cc. at *F*.

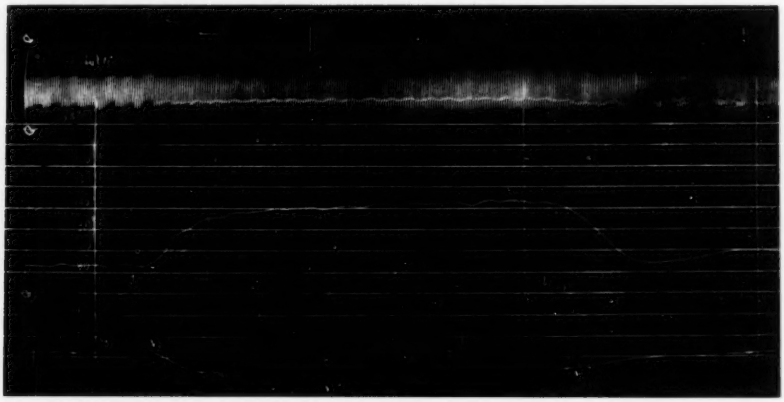


Fig. 3. Tracings showing effect of stimulating a central vagus nerve on spleen volume (lower), mean arterial pressure (middle), and stroke volume of the ventricles recorded by an oncometer and segment capsule (upper). Note changes in diastolic size (upper part of cardiometer record).

After an approximate 1½ hour period of severe hypotension the remaining blood was reinjected at *G* and the mean blood pressure rose to 135 mm. Hg. The pressure pulses were large and reasonably normal as regards contour. However, the spleen showed only a slight tendency to increase in size and never approached its control value. This mode of behavior differs materially from that observed during the reinfusion period in figure 1. Shortly after the reinjection the mean blood pressure started to fall, the pressure pulses became smaller and began to deteriorate. One hour later, when mean pressure had been reduced to 50 mm. the animal expired in respiratory failure. It is of interest to note that the spleen area slowly decreased somewhat more during this final period of progressively developing hypotension, but never significantly below the minimum value reached during the initial hemorrhage. This can be interpreted to mean that during hemorrhage the spleen contracted to its maximum ability and could react to no further stimulus of hypotension.

At autopsy the duodenal, jejunal and ileal mucosa was swollen, edematous, purplish and intensely congested. Hemorrhagic areas were present in the mucosa and the lumen contained extravasated blood. The characteristic sharp demarcation at the duodenal bulb described previously by others (2, 3) and by us (8) was present. The spleen was dry, pale and rough, with a few small irregular hemorrhagic areas beneath its capsule. No blood drained from the cut surface and little could be squeezed out by pressure.

The results of six other experiments were similar to those detailed above. In all cases the spleen area decreased rapidly and extensively during a hemorrhage sufficient to induce serious hypotension and contracted slightly more during the period of low tension. This indicates that, in the dog, the splenic contraction not only acts as a compensatory mechanism to place more blood into circulation during loss of blood volume but remains contracted during the period of post-hemorrhagic hypotension as well. When blood was reinjected the spleen enlarged somewhat, but never to control size, if the period of hypotension had not been too prolonged. Thereafter it slowly decreased again (cf. fig. 1) and apparently aided in sustaining the venous return and arterial pressure in animals that did not develop circulatory failure within three or four hours after reinfusion. In animals that developed hemorrhagic shock according to dynamic and pathological criteria, the spleen remained contracted during reinfusion (fig. 2) or increased very slightly for a short time. A dilated spleen, or one that approximated the control size, was never observed in these experiments.

Finally, we performed two experiments to determine whether greater concentration of blood might modify the results. To this end, the blood withdrawn was centrifuged and the corpuscles reinjected with a small amount of Locke's solution. A typical shrinking of the spleen occurred with each hemorrhage and a slight recovery (ca. 8–10 per cent) followed the injection of red cells. The injection of heparinized plasma after a period of hypotension sufficient to induce circulatory failure did not affect the spleen size; indeed, in one experiment it decreased still more toward the end, i.e., to 54 per cent of the control size.

Discussion. The dynamic significance of splenic contraction and its purpose in hypotensive states merit brief discussion. Changes in the size of the spleen have been frequently recorded in physiological and pharmacological investigations and several points of view have apparently developed as to the inferences which may be drawn from such changes in size.

One viewpoint appears to be that changes in spleen size permit conclusions regarding changes in peripheral resistance and particularly those in the splanchnic area. Our own experiences indicate that such deductions are not allowable. From a hydraulic viewpoint, the spleen circuit is only a minor shunt in the many parallel circuits between the aorta and venae cavae (cf. fig. 3, ref. 11) and, *a priori*, changes in its resistance could have no great effect on total peripheral resistance calculated as $1/R = 1/r_1 + 1/r_2 + 1/r_3 + 1/r_4 \cdots 1/r_n$, in which the spleen circuit could be any one of the fractions. The truth of this deduction can be demonstrated easily in a dog under barbital anesthesia, i.e., under conditions in which the spleen is large and congested and its vascular resistance would

presumably be low. Clamping the whole pedicle of such an exteriorized spleen, i.e., making the splenic resistance infinite—is without noticeable effect on mean blood pressure or on optically recorded pressure pulses. In a few unreported experiments by Dingle, Kent et al. (12) in which T.P.R. was calculated on the basis of cardiac output and mean arterial pressure, no change was found on clamping the splenic pedicle. The inference would appear to be that changes in resistance in the splenic shunt, like those in the limbs (13), are essentially without effect on total peripheral resistance in the whole animal.

The assumption made by many, but specifically in relation to shock by Moore (7), that significant decrease in size of the spleen is an index of intense vasoconstriction throughout the splanchnic area, seems rather hazardous on *a priori* grounds. In experimental animals, such as the cat and dog, changes in spleen size and vascular resistance to flow through the organ are chiefly, if not wholly, determined by action of extravascular muscle fibers in trabeculae, not to muscular elements in arterioles, as in the rest of the splanchnic circuit. To conclude, without evidence, that activity of such extravascular musculature is necessarily co-ordinated with vascular muscle tissue elsewhere is indeed an assumption. That it is not permissible seems clear from other observations from this laboratory (14, 15) that calculated T.P.R. may be reduced in certain forms of shock, although the spleen is always greatly contracted, postmortem.

As emphasized by Barcroft (9, 10), the chief function served by contraction of the spleen in the dog and cat is to demobilize blood, i.e., to cause an autotransfusion and so increase venous return. That this is sufficient to improve venous return, increase the diastolic size of the heart and augment systolic discharge is shown by experiments, one of which is reproduced as figure 3. In this instance, splenic contraction and elevation of arterial pressures were produced by stimulation of pressor fibers of the central vagus. Similar and even more marked effects follow injection of epinephrine, etc. In all such reactions, contraction of the spleen contributes to the elevation of arterial pressure not by the increase in resistance, but by augmenting venous return and increasing cardiac output.

Consequently, the development of splenic contraction during posthemorrhagic hypotension and its maintenance despite restoration of blood volumes by blood infusion indicates that in dogs splenic contraction is one of the important compensatory mechanisms invoked to increase the diminished venous return.

The fact that the spleen remains contracted when an irreversible circulatory state develops, and indeed remains so until death, exhonorates the spleen as a factor which precipitates the irreversible state in shock.

SUMMARY AND CONCLUSIONS

In nine experiments on dogs, the changes in areas of exteriorized spleens were studied by the method of Barcroft and Stephens during hemorrhage, posthemorrhagic hypotension and hemorrhagic shock following reinjection of the blood which had been withdrawn.

In confirmation of previous reports, it was found that the spleen contracts

rapidly and extremely during hemorrhage, the area being reduced by as much as 50 per cent or more. In extension, it was found that during a period of prolonged posthemorrhagic hypotension the dog's spleen undergoes a further slow contraction, does not increase on reinfusion of the withdrawn blood, but remains contracted whenever the duration and intensity of the hypotension is sufficient to create dynamic and pathological signs of shock. Similar changes occurred in plasmapheresis experiments.

In the dog, splenic contraction does not contribute to elevation or maintenance of arterial pressure by virtue of the increased resistance induced in the splenic shunt, but by augmenting venous return and cardiac output. The spleen is not an organ which withdraws blood from active circulation in hemorrhagic shock. When the spleen is found large and congested at autopsy other factors must have operated.

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A STUDY OF SPONTANEOUS FULMINANT SHOCK IN A HEART-LUNG-DOG PREPARATION[1]

RENÉ WÉGRIA, ALBERTO GUEVARA ROJAS AND CARL J. WIGGERS

From the Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio

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Previously, Wiggers and Werle (1) had attempted to assess the relative importance of changes in venous pressure, myocardial response and total peripheral resistance in hemorrhagic shock[2] by registering changes in arterial and venous pressures and in ventricular volume optically and by subsequently calculating total peripheral resistances (TPR). Results strongly suggested that, in different animals, depression of the myocardium and failure of peripheral resistance contributed in different degrees to the production of an irreversible state. However, changes in cardiac output primary to myocardial depression and secondary to altering venous pressures could only occasionally be dissociated during the course of such experiments. It seemed possible that such evaluation might be accomplished continuously by producing shock in a preparation in which venous pressure and cardiac output were under control of the experimenter. In addition, changes in total peripheral resistance (TPR) could be studied in a preparation in which cardiac ouptut per minute was measured by a different method. For these purposes, we devised a heart-lung-dog preparation.

The heart-lung-dog preparation. This preparation resembled Starling's heart-lung preparation as regards artificial control of venous pressures and cardiac output, but retained the natural circulation of the animal and kept operation of its natural cardiac and vasomotor reflexes intact. The principle (fig. 1) consisted *1*, in draining blood returned both by the superior and inferior venae (*SVC*; *IVC*) into a low-level reservoir of about 500 cc. capacity; *2*, in pumping this blood by a rotary pump, *P*, to a Mariotte bottle, *M*, the overflow of which returned to the low-level reservoir, and *3*, in feeding the right heart exclusively by blood at constant temperature from the Mariotte bottle. Venous inflow was measured periodically by a stromuhr of the Ludwig type, *S.U.*, and this flow was considered equivalent to left ventricular output. The stromuhr was actuated by large electromagnetic clamps in circuit with a chronoscope, thus eliminating possible variations in reaction times inherent in any manual method of timing flows.[3] The principles of the perfusion scheme and the registration of aortic pressure, *OM*, and venous pressure, *VP*, should be obvious from the sim-

[1] This research was supported by a grant from the Commonwealth Fund.

[2] Hemorrhagic shock was differentiated from hemorrhagic hypotension by the production of intensive congestion, edema or even hemorrhage in the mucosa of the upper intestine and failure of the circulation to respond more than temporarily to reinfusion of the blood withdrawn.

[3] We are indebted to Dr. Harold Green for aid in designing the electromagnetic stromuhr.

plified diagram of figure 1. However, a few technical details must be mentioned:

Experience soon showed that 2500 to 3000 cc. of undiluted, noncoagulable and compatible dog's blood were required for such shock experiments. Of this, 1000 cc. were needed to load the perfusion system. Hence, three large dogs were bled using 0.3 cc. liquaemin per 100 cc. as an anticoagulant for drawn blood. The experimental animal was given an initial injection of 0.3 cc./kilo liquaemin and, after perfusion had started, 0.1 cc./kilo was added to the reservoir every 30 minutes.[4] In some experiments, Calcomine fast pink[4], 80 to 150 mgm. per kilo of the total weights of all animals was added, in addition.

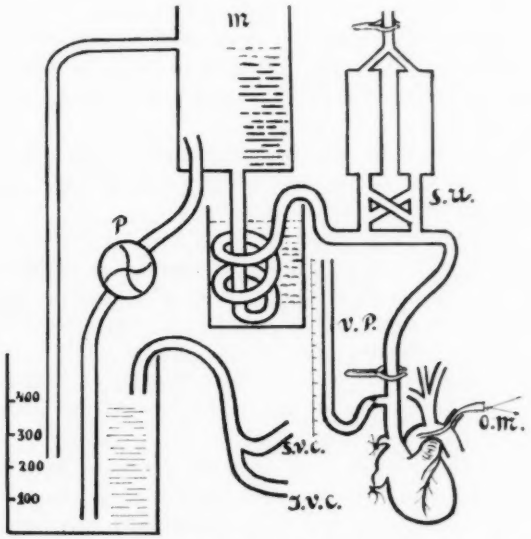

Fig. 1. Schematic diagram of heart-lung-dog preparation. Lettering explained in text. Not drawn to scale.

These exorbitant doses of liquaemin and dye were found necessary in order to preclude coagulation absolutely; smaller quantities invariably resulted in deposits of a fibrin on filters or caused infarct areas in the lungs, endocardium, mesentery or other organs.

The question at once arose as to possible deleterious effects of such enormous doses of anitcoagulants, particularly upon endothelial cells. Control injections of identical doses of liquaemin generally caused no hemodynamic changes; and only occasionally a temporary reduction of arterial pressures with a prompt recovery to normal. Similarly, the dye occasionally invoked temporary Traube-

[4] We are indebted to Roche-Organon Co., Nutley, N. J., for their generous contribution of the considerable quantities of Liquaemin solution required for these experiments and to Calco Chemical Co., Bound Brook, N. J., for a supply of Calcomine fast pink.

Hering fluctuations. After four or six hours, however, no signs of circulatory failure or pathological changes in organs could be discovered. The congestive or hemorrhagic changes in the intestinal mucosa characteristic of shock were never seen. Of course, when dye was used, the red color was generally distributed through tissues, serous fluids, bile, urine, etc. We must realize, nevertheless, that use of such enormous doses of anticoagulants may summate with other insults in making the capillaries more vulnerable to any tested shock-producing agent. They have, however, an advantage over defibrinated blood in that the blood used undergoes much less hemolysis and never evokes pulmonary edema.

The sequence of operative procedures in routine experiments was as follows:

Stage I. Cannulation of a femoral artery and vein—institution of a degree of artificial respiration which still allowed spontaneous contractions of respiratory muscles—opening of the chest without hemorrhage, meticulous attention being given to ligation of all vessels—exposure of the heart which was left within the pericardium—recording of mean femoral pressure—injection of liquaemin or liquaemin plus dye.

Stage II. Placement of a single ligature around the azygos vein, double ligatures around the inferior and superior cavae, and the left subclavian artery (for insertion of aortic manometer cannula).

Stage III. While femoral mean pressure was continuously recorded, the following procedures followed as quickly as possible: *a*, ligation of the inferior vena cava near the right auricle and insertion of a cannula into its peripheral end, allowing blood to drain into the low-level reservoir;[5] *b*, ligation of the superior vena cava peripherally and insertion of cannula into the vein near the right auricle—commencement of artificial perfusion of heart—insertion of a cannula into the peripheral end of the superior vena cava and drainage of its blood into the low-level reservoir; *c*, ligation of the azygos vein.

With ligation of the inferior vena cava mean arterial pressure fell approximately to 40 mm. Hg and on subsequent ligation of the superior cava to 15 or 20 mm. Hg. The heart continued to beat regularly but feebly on the small supply from the azygos vein until the perfusion was started. Artificial venous inflow and pressure were slowly increased until arterial mean pressure oscillated around 120 mm. Hg. The total duration of significant hypotension (50 mm. Hg or less) required for transfer to an artificial circuit varied from five to nine minutes; in most experiments it was 5 to 7 minutes.

Optical manometers were inserted after the perfusion system was operating properly. The temperature of the blood was kept constant at 36° to 38° in different experiments by a thermostatic control of the water bath surrounding the warming coil. Changes in temperature due to variation in flow were quickly rectified or anticipated by addition of cold or warm water to the bath and resetting of its thermoregulator. Tests on a number of preparations showed that vasomotor and cardiac reflexes were active during the start and course of the experiment (e.g., central vagus stimulation and carotid compression).

[5] As a rule, the animal yielded about 250 cc., ca 25 per cent, of its calculated blood volume by the time artificial perfusion was started.

Expectations and realizations. It was our expectation that, after the technique had been mastered, such a preparation would maintain an unaltered status for a number of hours and that the rôle of peripheral factors, venous pressure and cardiac impairment could be assessed when shock was willfully produced in different ways. We discovered, however, that even under the most propitious circumstances, our animals quickly showed clear evidences of a rapidly developing shock, such as deterioration in the form of arterial pressure pulses, consumption and storage of large quantities of the blood perfusate and exhibition of marked congestive and hemorrhagic changes in the upper intestines. In short, it was only necessary to follow the changes which occurred spontaneously. The process progressed so rapidly as to constitute a *fulminant type of shock.*

Such experiments stress the fact that shock is not necessarily a slow progressive chain of events, but under propitious circumstances can develop with extreme celerity and intensity. Perhaps technical improvements, not obvious to us, could still be made which would prevent development of such a rapid peripheral circulatory failure; but since each experiment involved the use of four dogs and large quantities of heparin, we chose to discontinue these studies with the completion of twenty-four such experiments.

Results. Before discussing illustrative experiments, the initial circulatory state of our animals should be assessed. Our experimental procedures previous to perfusion had been so far refined that our animals, with open chests and under artificial respiration often had mean pressures of 150–170 mm. Hg and pressure pulses suggestive of a mild hypertension. After the brief period of hypotension incident to a change from the natural to the artificial blood supply, venous pressures and cardiac output were so adjusted that mean arterial pressures of 100 to 140 mm. Hg were re-established. The venous pressures and cardiac output required to accomplish this varied considerably in different animals of approximately the same weight. This is shown in table 1.

Such outputs are definitely below values usually accredited to dogs of these sizes. The amplitude and form of aortic pressure pulses before and after artificial perfusion also indicated that the systolic discharge was less than previous to perfusion. Since the normal dog's heart has a larger cardiac output at effective venous pressures of 50 to 60 mm. H_2O than the best in our series and since some of our hearts delivered still smaller volumes per minute at even higher venous pressures (cf. expts. 4, 8, 13, 14), it would be a fair inference that such artificially perfused hearts are below par at the start. This of course applies to any heart-lung preparation. On the other hand, in all experiments, vigorous regular beats were resumed at once and the right ventricle appeared full but not dilated; in short, the hearts seemed in good condition by every observable criterion and, during initial periods of observation, responded with constant increments and decrements of output to definite elevations and reductions of venous pressure. In short, the myocardial depression which appeared with artificial perfusion was stabilized, or, in some instances, tended to decrease during the control period.

Since mean pressure was sustained at 120 mm. Hg, or above, on such small

cardiac outputs, and an inordinate hypertension developed when cardiac output was significantly increased, it is a fair inference that these animals were in a state of superconstriction at the start. This is also supported numerically by

TABLE 1

EXPT.	WT.	V.P.	C.O/MIN	T.P.R.	B.P.	EXP.	WT.	V.P.	C.O/MIN	T.P.R.	B.P.
	kgm.	*mm. H_2O*	*cc.*	*A.U. $\times 10^3$*	*mm. Hg*		*kgm.*	*mm. H_2O*	*cc.*	*A.U. $\times 10^3$*	*mm. Hg*
1	12.5	105	1167	9.6	140	10	12	60	882	10.15	122–114
										11.05	
2	13.0	83	1220	9.5	130–145						
				8.55		11	14.5	85	892	8.8	100–112
										10.0	
		52	848	12.66	125–135						
				11.75		12	12.5	92	994	10.46	130
						13	10.5	80	616	14.25	110
3	14	52–56	1017	10.54	120–135						
				9.43				120	855	11.02	122
4	14	60–65	781	13.2	115–130	14	10	108	906	7.67	87
				11.6							
						15	13	70	1055	9.1	120
5	14	32	608	18.29							
				13.13	100–140			50	658	12.0	100–112
										13.5	
6	12.5	43	527	18.0							
				15.17	100–120	16	16	67	1120	8.55	120
7	14	55	1139	8.22	102–118	17	12.5	60	650	13.75	112–122
				7.1							
8	13.5	66	743	11.09	92–105						
				9.7							
9	12	65	991	9.23	107–115						
				8.6							

the rather high values obtained when total peripheral resistance (TPR) was calculated in absolute units (A.U.) according to the formula:

$$\text{TPR} = \frac{\text{mean pressure} \times 1332}{\text{cardiac output/sec.}} = \frac{\text{dynes} \cdot \text{sec.}}{\text{cm.}^5} \text{ (A.U.).}$$

While it is hazardous, at the current state of our knowledge, to draw too many conclusions from comparisons of calculated total peripheral resistance (TPR) made in different groups of experimental dogs, table 2 may have some interest.

This compilation indicates that the ranges of TPR calculated in this series are the highest of any reported series, and as shown in table 1, most of these

are near the upper ranges. The magnitude of the differences may be more intelligible when it is recalled that Dingle, Kent et al. (3) reported an experiment in which an increase in TPR from approximately 6000 to 15,000 A.U. represented the degree to which TPR augmented after a dose of 2 cc./1:50,000 adrenalin in a vagotomized dog. On the other hand, it should also be mentioned that, in repeated tests, stimulation of the central vagus or injection of neosynephrine, with constant cardiac output, were capable of increasing TPR enormously, showing that regardless of the high values they were certainly not maximal (see fig. 4).

While the significance and interpretation of changes in TPR, calculated in this customary fashion, deserve further study we are, for the time, accepting the interpretation that our preparations probably started with an intensive vasoconstriction and high TPR.

Shock without reduction in venous pressure. It is commonly postulated that all forms of shock have one feature in common, viz., initiation by a primary reduction in venous return. While there is substantial proof for the correctness of this doctrine in most forms of shock, some evidence exists that reduced arterial resistance may be the primary factor in certain types (for review and discussion, see 4). We shall first discuss representative experiments

TABLE 2

Böger (2)	4020–4470 A.U.*	probably small dogs
Dingle, Kent et al. (3)	3750–11,300 A.U.	(8–12 kilos)
Wiggers and Werle (1)	3931–11,714 A.U.	(10–15 kilos)
This series (see table 1)	7100–18,290 A.U.	(11–13 kilos)

* A.U. = absolute units in dynes. sec./cm^5.

which indicate that such a sequence occurs in the type of experiment which we designed.

Figure 2 graphically summarizes observations on a 14 kilo heart-lung-dog preparation in which right auricular pressure was kept constant at 55 mm. H_2O and the temperature of the blood remained at 37.5° throughout the experiment. The vagus nerves and the carotid arteries were intact. The period of hypotension caused by transfer of the heart from the natural to the artificial blood supply lasted six minutes. Ten minutes after artificial perfusion (start of plot) the mean pressure was maintained at 118 mm. Hg by a cardiac output of 1200 cc./min. The heart rate was 160/min. During the first half-hour this mean pressure was approximately maintained; but cardiac output slowly decreased to 1070 cc./min., while the calculated total peripheral resistance (TPR) increased a little.

Approximately at the end of 45 minutes, *B*, the venous pressures started to rise spontaneously, reaching 120 mm. H_2O, but cardiac output was not affected. This passive congestion indicated depression of the myocardium, a feature occasionally noted in other experiments. When no adjustments were made, the heart continued to discharge essentially the same volume under the influence of these greater pressures. Since it was the purpose of this experiment to main-

tain a constant venous pressure, it was necessary to decrease the venous inflow rate. This caused a reduction in cardiac output to 800 < 860/cc. per minute, *C*. An inspection of the graph shows, however, that once reduced to 71 per cent of its control value, cardiac output remained essentially constant for the remainder of the experiment.

With the slight decline of cardiac output during the early period, *A*, *B*, and the greater abrupt reduction at *C*, mean arterial pressure dropped correspondingly. After *D*, a slight recovery to 71 mm. Hg occurred. The pressure pulses —selected samples of which are shown on the lower part of the chart—altered

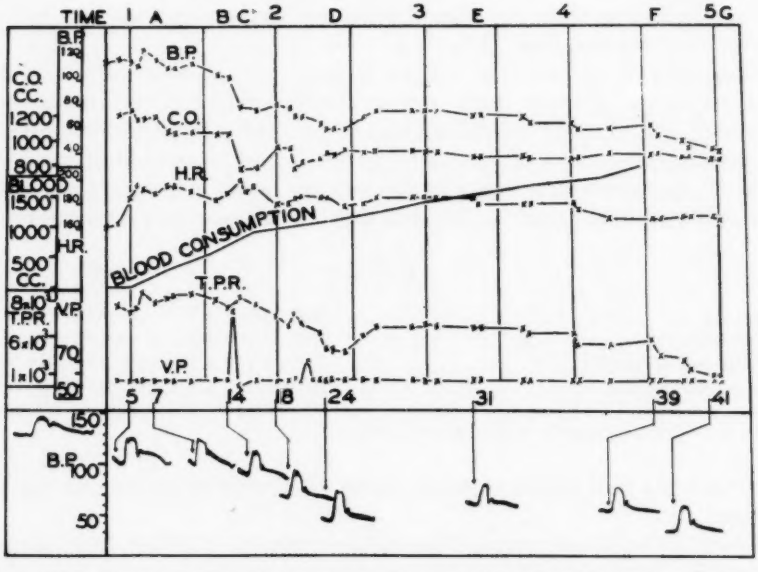

Fig. 2. Plot showing sequential cardio-circulatory changes under conditions described in text. *BP*—mean arterial pressure; *CO*—cardiac output per minute; *HR*—heart rate; *TPR*—total peripheral resistance in absolute units; *VP*—venous perfusion pressure. Below, transcribed aortic pressure pulses showing changes in form and in systolic and diastolic pressure.

in form and decreased in amplitude (curves *5–14*), much as during a large hemorrhage (6). With unchanged TPR, these changes were solely due to decreased systolic discharge. The changes in form and amplitude observed after large hemorrhages may also be due dominantly to reduction in systolic discharge.

While cardiac output remained reduced but constant during the period *D-G*. the heart slowed somewhat and therefore systolic discharge increased a little. The mean pressure nevertheless declined slowly from 71 mm. to 42 mm. during this period. This was solely due to a progressive decline in TPR which became much further reduced at a late stage, *F-G*.

With the early development of declining TPR, the pulse pressure increased

according to rule (curves *14–24*), but as it declined further, pulse pressure became smaller again (curves *31–41*). This illustrates the difficulty of assessing changes in peripheral resistance by pulse pressure variations, probably because a number of variables, such as reflected waves, also affect the pulse amplitude.

In the charts of various figures, the curves labeled "blood consumption" depict the rate at which blood was added to our reservoirs and hence the rate at which it was taken out of circulation by the dog. In the experiment plotted as figure 2 such storage began almost immediately and continued at so fast a rate that at the end of the first hour (2:00 p.m.) a liter of blood had been added to the perfusion system. Subtracting some 250 cc. returned by the animal during the early period of hypotension, the dog had nevertheless added to his own calculated blood volume of 980 cc. another 750 cc., within an hour. During the remainder of the experiment, during which a lower arterial pressure persisted, the rate of blood consumption was somewhat less but progressive, so that at the end the animal had sequestered a total extra blood volume equal to 1900 cc., or almost three times its own blood volume.

Since the quantities thus stored sometimes reached three liters, experiments of this nature offered a unique opportunity for discovering the places in which it is sequestered. The autopsy findings were fairly typical. There was no obvious increase in peritoneal or other serous fluids nor gross evidence of pulmonary congestion or edema. The kidneys, liver and pancreas were not congested and, with two exceptions, the spleen was small and contracted. The leg muscles were dry and not edematous. However, the small intestines from the middle of the duodenal bulb to the lower ileum were swollen and, on opening, the mucosa were always found to be intensely congested and purplish in color. Colored photographs were almost identical with a plate published by Whipple et al. (5) illustrating changes produced by injection of filtered intestinal contents from animals with intestinal obstruction. Hemorrhagic areas and blood in the lumen were not uncommon findings. There seems to be no question that the excess blood was almost wholly stored in the intestinal mucosa, as first reported by Erlanger and Gasser (7) in other forms of shock, and consistently noted by us (6) in hemorrhagic shock. No post mortem evidence of generalized capillary damage in other organs, such as Moon (8) has described, could be found. Only one other pathological change of consequence was noted fairly constantly, viz., superficial subendocardial hemorrhages varying in extent, intensity and distribution. In this experiment they were limited to the right ventricle, in the experiment next analyzed to the left ventricle, and occasionally both chambers were affected. No electrocardiographic changes were associated with these subendocardial hemorrhages. Such hemorrhages were never noted by Werle, Cosby and Wiggers (6) in autopsies on dogs that had died from hemorrhagic shock.

In summary, this, like several other experiments of our series, may be said to simulate experiments on an intact animal in which a fulminating type of shock develops as a result of a progressive decrease in total peripheral resistance, but in which venous pressure remains or is kept constant. The thought suggests

itself that maintenance of a normal venous pressure may not be a satisfactory method for combating such types of shock.

A somewhat similar course of events developed when venous pressure and cardiac output were materially increased to values sufficient to maintain normal or high arterial pressures at the start. Thus, in two experiments, cardiac output volumes of about 1500 cc. maintained mean pressures of 148 and 162 mm. Hg respectively, but arterial pressures fell abruptly during the first half-hour and the animals at once started to consume large volumes of extra blood from the reservoir. Indeed, it soon became obvious that maintenance of large initial cardiac outputs and high initial arterial pressures did not prevent or retard, but rather hastened, the downward trend of arterial pressures and accelerated the consumption of blood.

Shock with maintained or increased cardiac output.

Figure 3 illustrates data from an experiment in which attempts were made to maintain arterial pressures by progressively increasing the venous pressure and cardiac output. The plot of mean arterial pressures, *A*, *B*, illustrates the mode of recovery from a previous six minute period of hypotension (40 > 20 mm. Hg). At the very onset, a venous pressure of 60 mm. and cardiac output of only 630 cc./min. sufficed to maintain mean arterial pressure at 110–120 mm. Hg for about 35 minutes, *A-C*. Shortly thereafter TPR began to decrease rapidly and arterial pressures declined abruptly. The pressure pulses (cf. curves *10*, *11*, *14*) increased in amplitude according to rule, the summit became peaked and the diastolic limb following the incisura was flattened. The blood consumption curve shows that during this preliminary period comparatively little blood had been consumed.

During the observations made at the time indicated by *C* on the plot it was noticed that cardiac output was beginning to decrease a little, hence venous pressure was raised to 67 mm. H_2O to compensate for this reduction. This had little effect on arterial pressures or pressure pulses. At *F*, venous pressure was further raised to 90 mm. H_2O in an effort to maintain arterial pressure by augmenting cardiac output to 1020 cc./min. This represented a 61 per cent increase over the initial output at *A-B*. Through this expedient mean arterial pressure increased to 90 mm. at *E-F*; subsequently fell a little, *F-G*; but toward the end declined rapidly, *G-H*. The pressure pulses remained large and collapsing (curve *33* to *65*).

During the initial decline of arterial pressure (fig. 3, *B-F*), TPR decreased significantly, actually from about 15×10^3 to 7.2×10^3 A.U.; from *F* to *G* it stabilized and, thereafter, decreased to a final low of about 5×10^3 A.U. Attempts to evoke a pressor reaction by stimulation of both vagus nerves with a strong tetanizing current failed.

Attention should be called to the fact that with increase in cardiac output at *F*, the rate at which blood was withdrawn from circulation increased and that this was maintained fairly constant until the end, when 3.2 liters of additional blood or an amount equal to one-fourth of the dog's body weight had been stored.

The autopsy findings again showed no increased blood storage in any other organ than the intestinal mucosa, but this extended to branches of the mesenteric vessels, many of which appeared to be thrombosed.

The time-curve of the mucosal changes was studied during the course of several experiments. In this instance, examination of a duodenal loop temporarily exteriorized at 1:10 p.m. revealed no obvious pathological changes, and a second inspection at 3:05 p.m. of a segment 4 or 5 cm. lower also showed no outstanding congestion, despite the fact that the animal had demobilized nearly 1.5 liters of blood. Apparently the pathological congestive changes at

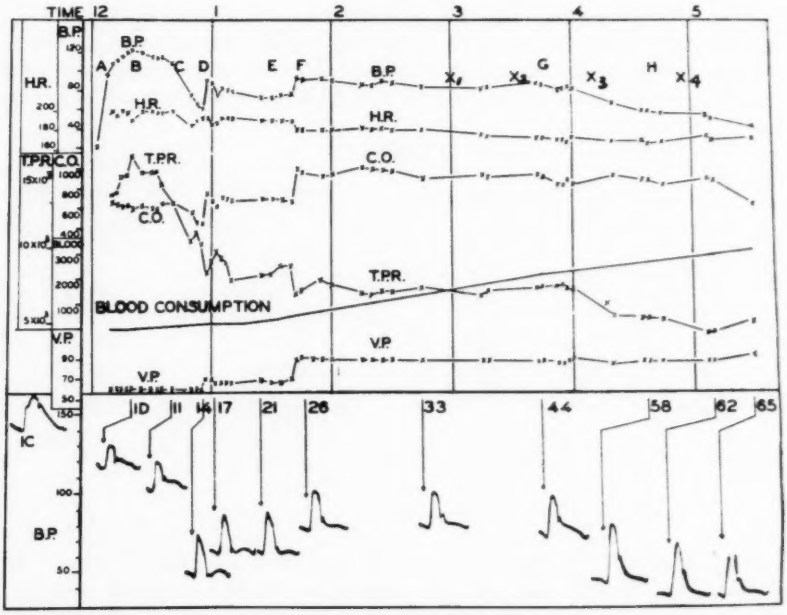

Fig. 3. Plot showing sequence of cardio-circulatory changes under conditions described in text. Designation of curves as in figure 2.

autopsy are not occasioned by a mere mechanical packing of blood into the vessels but seems to require some specific change in blood vessels. This correlates with observations made previously (6) that mucosal changes also occur when there is less blood in the body after prolonged hemorrhagic hypotension. At 4:10 p.m. a third inspection of a segment 4 cm. above the first revealed the characteristic pathological picture. The occurrence of such intensive mucosal changes, despite maintenance of a good cardiac output and arterial pressure, indicates that early deprivation of blood supply, anoxia, etc., is not the only factor concerned with the vascular changes. Since TPR decreased significantly, it could not have been due to vasoconstriction. Maintenance of an arterial

hypotension at 20 mm. for 6 to 8 minutes in an intact dog through hemorrhage also does not produce these changes.

Summarizing this and similar experiments, it is demonstrated that conditions can be created experimentally in which all the dynamic and pathological criteria of shock are produced speedily despite the fact that venous pressure and cardiac output are increased by 50 per cent or more. The causative mechanisms lie wholly in the periphery of the arterial tree. It is also shown that, except toward the end, cardiac output at equivalent venous pressures decreases but little. This was true not only at the pressures plotted, but at temporarily lower venous pressures tested at the intervals marked X^1 to X^4. To prevent confusion these were not indicated on the plot, but are briefly summarized in table 3.

While there is a slight reduction in cardiac output at equivalent venous pressures this proved no greater than may occur in such artificial preparations even though the coronary vessels are fed by a substantial pressure head in the aorta.

TABLE 3

CONTROL AT	VENOUS PRESSURE	CARDIAC OUTPUT PER MIN./CC.	PERCENTAGE OF ORIGINAL OUTPUT
	mm. H_2O		
E-F	90	1020	100
	67	630	100
X^1	90	925–946	92.7
	68	608–613	97.3
X^2	90	919–943	92.4
	68	502–507	90.0
X^3	90	841–891	87.3
	68	507–542	86.0
X^3	90	870–928	91
	70	610–612	97.1

Finally, it should be stated that in the particular experiment chosen as an illustration (fig. 3), the dog had been primed on the previous day with 10 cc. cortical adrenal hormone (Eschatin) and with 20 cc. on the morning of the experiment. In addition, 10 cc. was administered during the artificial perfusion. Since the course of events did not differ from that of untreated dogs, the data from this experiment serve at once to illustrate the natural course of events and to place on record details of one of our negative experiments with adrenal cortical hormone. The results of other experiments are analyzed later in this communication.

Shock with reduced venous pressure and diminished cardiac output. Experiments so far discussed did not duplicate conditions admittedly found in shock, viz., reduction in venous return and secondary decrease in cardiac output. Most of our experiments were naturally designed to simulate such conditions. This could easily be done in our preparation by merely reducing the venous return through lowering of the Mariotte perfusion bottle.

The general procedure and nature of results are illustrated by an experiment

performed on a 13 kilo dog and charted as figure 4. During the control period, *A-B*, a venous pressure of 70 mm. H_2O and a cardiac output of 1055 cc. per minute maintained an arterial pressure of about 120 mm. Hg. After control data had been collected during a 20 minute interval, venous pressures were reduced in two steps, viz., to 50 mm. H_2O at *B* and to 32 mm. H_2O at *C*. This simulated the effects of two large hemorrhages, and in fact an excess volume of about 200

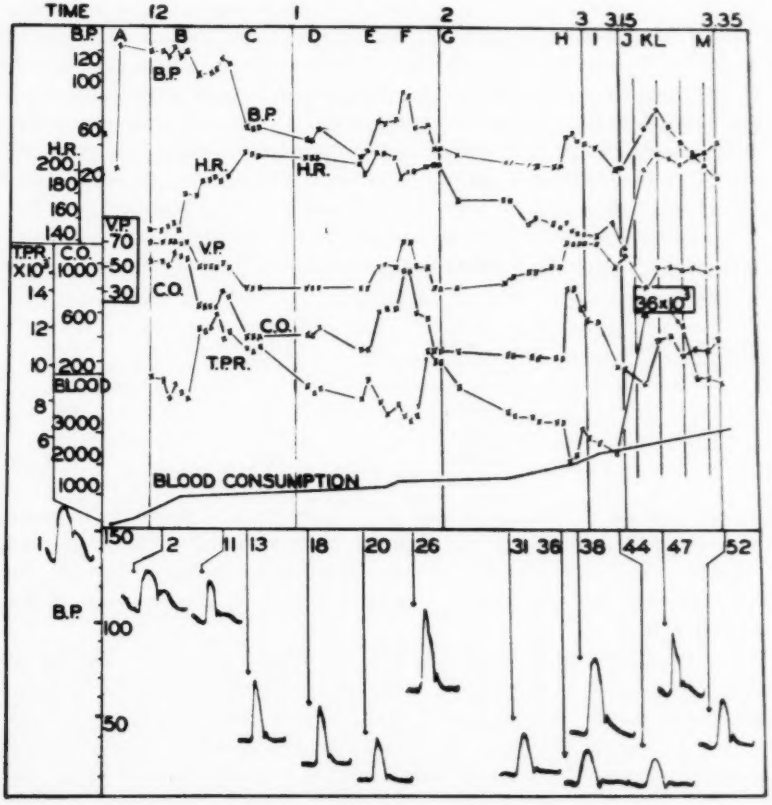

Fig. 4. Plot showing sequence of cardio-circulatory changes under conditions described in text. Designation of curves as in figure 2.

cc. was returned from the animal to the lower reservoir. With the initial decrease in venous pressure and cardiac output at *B*, the heart rate accelerated and TPR increased, as also happens promptly after hemorrhage. With the second reduction at *C*, however, TPR returned to normal and thereafter the progressive trend was distinctly downward.

Selected pressure curves during the early stages (*A-C*) show an increase in amplitude, a peaked and collapsing form and a characteristic flattening of the

diastolic limb (curves *2*, *11*, *13*). Had such curves been obtained in an intact animal, the temptation would have arisen to attribute these phenomena to an initial reduction of TPR. However, in these experiments calculations showed that TPR was higher than the control. Apparently, circumstances can arise during which deductions concerning the circulatory state may not be drawn from pressure pulses on the basis of theoretical deductions or experiments on artificial models.

During the control interval, *A-B*, the net consumption of blood was about 700 cc. After reduction of venous and arterial pressures the blood storage during the period, *C-E*, continued steadily but at a slower rate. Mean arterial pressure slowly declined from 60 to 38 mm. Hg, due almost wholly to a decreasing TPR, and not significantly to reduced cardiac output. At this time it was deemed expedient to determine the cardiac output responses to higher venous pressures (50 and 70 mm. H_2O) used earlier in the experiment. As can be seen at *E*, *F* on the chart, cardiac outputs at these pressures were somewhat less than at *A*, *B*, *C*, but not sufficiently so as to indicate any inordinate depression of the myocardium. It may be pointed out, parenthetically, that with the purposeful

TABLE 4

VENOUS PRESSURE	C.O. AT B-C	C.O. AT E-F	C.O. AT H
mm. H_2O	*cc.*	*cc.*	*cc.*
70	1055	940	685
50	658	620	253
32	406	300	272

two-stage increase in cardiac output *E-F*, mean arterial pressures increased to 80 mm. Hg, but the form of the pressure pulse retained its collapsing feature (curve *26*). Obviously, the rise in mean pressure following an increase in cardiac output does not necessarily signify a degree of improvement ordinarily associated with it.

At *G*, the experiment was continued at low venous pressure. During the 50 minute interval indicated by *G-H*, mean arterial pressure and TPR decreased progressively, cardiac output slowly declined and pressure pulses decreased in amplitude (curves *26*, *31*, *36*). The heart rate now slowed progressively despite constancy of temperature, a sign which seemed to be associated with onset of myocardial failure in hemorrhage experiments reported by Werle, Cosby and Wiggers (6). At *H*, the cardiac output at 32, 50 and 70 mm. H_2O was again tested. Table 4 shows some comparative data at equivalent venous pressures.

Following these tests, *I*, venous pressures could not be reduced below 51 mm. H_2O; even at this pressure cardiac output declined progressively to 170 cc./min. All signs indicated that progressive myocardial depression contributed significantly to the progressive decline of arterial hypotension and that elevation of venous pressure following an increase in venous return did not restore cardiac output.

Analyzing fourteen such experiments, we feel justified in concluding that in

preparations in which a hypotension of 50 mm. Hg or less lasts for 1 to 1.5 hours, depression of the myocardium occurs, in the sense that its capacity to eject equal blood volumes at equivalent venous pressures is decidedly reduced.

The effects of neosynephrine at terminal stages. During the terminal stages of five experiments we tested the cardiac and vascular responses to neosynephrine and their effect on the course of events. Tests earlier in the course of our experiments appeared inadvisable; they might have obscured the natural course of events. Neosynephrine was chosen, because the pressor effects are more sustained than those of epinephrine and other pressor amines of the catechol group, and the doses necessary to produce a given pressure response is less than for other members of the phenol group of pressor amines. Furthermore, according to Crismon and Tainter (9), its pressor potency is greater in circulatory depression following destruction of the brain and medulla than it is in normal animals.

A typical reaction following addition of 0.25 cc. of a 1 per cent solution of neosynephrine[6] to the lower blood reservoir is shown in figure 4 from *J* to *M*. Mean pressure increased rapidly from 20 to 70 mm., the slowed heart accelerated and TPR increased so tremendously that it is indicated numerically (36×10^3 A.U.). Such reactions certainly proved that the capacity of peripheral vasoconstriction has not been lost in our preparation. The blood pressure reactions resembled those obtained on animals after section of the spinal cord or destruction of medullary centers (9). The venous pressure initially fell, *J*, *K*, indicating a temporary increase in cardiac output between flowmeter readings; but, as the venous pressure decreased, the metered cardiac output at *K* was actually less. Thereupon, we increased venous inflow sufficiently to restore venous pressure to the previous level, *L*. As a result, cardiac output augmented. Comparison of pressure pulses 44 and 47 show, however, that a natural form was not restored as arterial pressure rose.

During the following 15 minutes, *L*-*M*, the vascular effects gradually waned; TPR dropped but remained above previous levels, as did the blood pressure which had a terminal value of 40 mm. Hg. The after-effects on the heart, possibly due to temporary improvement in its blood supply were more persistent. The heart remained rapid and cardiac output did not decline. The final pressure pulse (curve *52*) shows a definite improvement when compared to that of curve *44* taken previous to use of the drug.

The effects of cortical adrenal hormones. When a sufficient number of experiments had been performed to convince us that the dynamic and pathological intestinal changes could not be prevented by experimental expedients, it occurred to us that this preparation offered a supreme opportunity to test the value of cortical adrenal hormones. If the adrenal hormones could prevent or retard the fulminating circulatory collapse and avert or ameliorate the damage to the intestinal mucosa, their value would be demonstrated beyond all doubt; but we, of course, recognized from the start that failure to achieve such results would

[6] We are indebted to Frederick Stearns and Co., Detroit, for the neosynephrine utilized in these experiments.

not necessarily establish their inefficiency in less violent and more slowly progressing forms of shock.

Three dogs were treated with the commercial preparation, Eschatin,[7] containing 25 dog units per cubic centimeter, and two dogs with a preparation of whole adrenal cortex (702)[7] representing 50 grams adrenal cortex per cubic centimeter. The dogs received a large priming dose early in the morning before the experiment, and repeated additions were made to the blood reservoirs during the experiment. Two dogs received an additional intramuscular priming dose the afternoon before an experiment. Table 5 shows that the total doses given are many times those necessary to support an adrenalectomized animal.

A survey of our results, either as regards the course of dynamic events or the pathological findings in the intestinal mucosa, fails to disclose evidence of any beneficial influence. One of the experiments has already been described in detail.

DISCUSSION. *The rôle of myocardial depression.* Suspicions that some degree of myocardial depression occurs during shock have been voiced by a number of

TABLE 5

EXP.	PRIMING DOSES (INTRAMUSCULAR)	TOTAL DOSE	PREPARATION
	cc.	*cc.*	
1	5	10	Eschatin
2	2.5	8.5	Eschatin
3	3.5	20	Kendall preparation
4	10*	20	Kendall preparation
5	10*	40	Eschatin

* 5 cc., previous afternoon; 5 cc., early morning of experiment.

investigators, among them Howell (10), Y. Henderson (11) and Erlanger and Gasser (12). In recent reports from this laboratory (1, 6) it was suggested that its importance had perhaps been minimized in recent years. However, the question whether it *a*, is concerned in the initial reduction of cardiac output and initiation of shock; *b*, acts solely as a contributing factor; *c*, is a factor in development of an irreversible circulatory state, or *d*, causes the terminal circulatory failure, remained in a speculative stage.

In the type of experiment here reported, the myocardial depression which follows prolonged extreme hypotension is a comparatively late phenomenon which appears definitely after the process of blood sequestration is well under way and the intestinal mucosa has become extensively congestive and hemorrhagic.[8] In comparison with intestinal vessels, the myocardium and central

[7] We are indebted to Parke Davis and Co. for the supply of Eschatin, and to Dr. E. C. Kendall for the preparation no. 702 used in these experiments.

[8] Obviously, this deduction is predicated on the supposition that the initial depression of the myocardium which apparently accompanies the transfer to an artificial perfusion system (p. 215) is not a part of the shock picture. This could be argued, but we have adopted the view which is least favorable to a hypothesis that myocardial failure is of importance during the development of shock.

nervous system show a remarkable degree of resistance to shock-producing agents. Myocardial depression is therefore not an initiating factor. Irreversibility also is established before myocardial depression sets in.[8] However, there is no question that when peripheral processes have advanced considerably, myocardial depression contributes to the suddenness of the circulatory failure, and when failure of the respiration does not occur previously it is responsible for the terminal circulatory failure.

The significance of reduced total peripheral resistance. In previous studies Wiggers and Werle (1) noted that, during and after development of hemorrhagic shock, the calculated TPR decreased to or below control values. Since the state of vascular resistance at the start of observations remained unknown and the magnitude of changes required to make a diagnosis of reduction in TPR had not been established, we made the more conservative statement that TPR is not increased in every form of shock, as is currently believed.

In experiments now reported, there can be no doubt that the significant and constant changes during our observation periods consisted in a steady and pronounced decrease in TPR. Whether this was of central or peripheral origin we are not sure.

Such results do not contradict the continuance of vasoconstriction in structures of the limbs, for as one of us (13) has analyzed, changes in splanchnic resistance apparently dominate total peripheral resistance. Nor can these observations necessarily be interpreted as opposed to a generally accepted view that generalized vasoconstriction is the dominant characteristic during early stages of shock. As previously pointed out (4), many contradictory interpretations regarding the mechanism of shock which occur in the literature have been due to the fact that differences in experimentation or in the condition of the animal at the start were not always scrutinized carefully. We must guard against such pitfalls to the best of our ability.

Again reviewing our results, it should be emphasized that in some of our experiments, as illustrated by that of figure 3, TPR increased initially. In that larger number of experiments in which a decrease in TPR developed progressively from the start it could be assumed that the phase of intensive generalized constriction occurred previous to our observations and, further, that shock-inducing processes had started to work during the operative procedures which preceded. The apparent existence of an increased TPR at the start of our experiments harmonizes with such a view. Since most experimenters agree that the preliminary phase of intense vasoconstriction during shock is followed by a phase of reduced peripheral resistance, it is possible that in our experiments the phase of increasing TPR was obscured and the later phase of decreasing TPR was made especially prominent.

Consequently, while we cannot be certain from our experiments whether increased or decreased TPR were initiating factors, it seems clear that development of an irreversible state correlates with a reduction in TPR.

Applicability of results. It is obviously necessary to apply our results and interpretations to other forms of shock with the greatest care. It is conceivable that the clear-cut results obtained in our preparation may have started us on a

false lead, as regards general applicability or, indeed, applicability to any form of shock. It may again be pointed out, however, that contrary deductions regarding the state of peripheral resistance when true shock has begun to develop are not based on rigid application of experimental data (cf. Wiggers, 4). No one has yet succeeded in evaluating or following changes in TPR in intact shocked animals. Only when contrary proof has been adduced by such studies can it be stated with certainty that reduction in TPR is not the factor which determines irreversibility, without which a state of true shock can probably not be recognized. For ourselves, we are prepared to change our viewpoint repeatedly, meanwhile recording experimental facts from time to time regardless of whether they will eventually prove to have or not to have a practical bearing.

In conclusion, we would re-emphasize that while reserve and caution must be exercised in applying the results of these experiments to forms of shock which develop more slowly, they may have a bearing on more fulminant types, not entirely unknown clinically. Every victim of a serious accident, not immediately killed, who is transported to an emergency station *in extremis* and expires within a few hours despite routine efforts to save his life must be suspected of having died from a fulminant type of shock. This is but one of many clinical examples which suggest themselves. Too little attention has perhaps been given both to the diagnosis of fulminant shock by clinicians and to its study and analysis by investigators. The possibility that fulminant shock arises as a result of a rapid development of the phase of reduced total peripheral resistance and may require a different routine management must also be considered.

SUMMARY

1. The relative importance of *a*, decrease in venous pressure and secondary reduction of cardiac output; *b*, primary myocardial impairment, and *c*, changes in total peripheral resistance (TPR) in shock was studied by means of a heart-lung-dog preparation, which is described. Venous flow and cardiac output were under control and TPR could be calculated. In such a preparation, a fulminant type of shock developed spontaneously which may resemble clinical types characterized by speedy circulatory failure and death.

2. In twenty-four such experiments it was shown *a*, that shock can develop without progressive reduction or even with a rise of venous pressures or cardiac output, and *b*, that it cannot be prevented or cured by increasing venous inflow and cardiac output.

3. The fundamental factor responsible for this type of irreversible circulatory failure was a steady and pronounced decrease in total peripheral resistance, which enabled the animal to store, over and above its own blood volume, quantities of blood equal to 25 per cent of its body weight or four times its own blood volume. Autopsies revealed no storage depots other than the mucosa of the upper intestines which was always edematous, intensely congested and often hemorrhagic. The integration of these observations with apparently contrary findings regarding the state of the peripheral circulation, and their application to fulminant types of shock in man are discussed.

4. Following any prolonged period of hypotension during the development of shock, the cardiac output at equivalent venous pressures also decreased, indicating that depression of the myocardium occurred. In the form of shock studied, this did not prove to be an initiating factor nor was it necessary to produce an irreversible state. But, after the peripheral changes of shock had been well established, it played an important rôle in the rapid downward trend of blood pressure and was the ultimate cause of death.

5. Administration of neosynephrine during terminal stages evoked a tremendous increase in peripheral resistance and increased cardiac output at equivalent venous pressures, indicating that the power of vascular constriction was not lost and that the myocardium was stimulated. While this resulted in a marked increase in mean arterial pressure and apparent improvement lasting over half an hour, the optical pressure pulses had a peaked summit and collapsing character, proving that dynamic improvement cannot be judged by elevation of mean pressure alone.

6. Five dogs primed with liberal doses of cortical adrenal extracts and receiving these extracts during the experiments, revealed no indications that the course of dynamic events or the pathological changes in the intestinal mucosa were influenced. It must be recognized, however, that our preparations perhaps offered too severe a test.

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INCREASED ERYTHROCYTE DESTRUCTION ON A HIGH FAT DIET[1]

ARTHUR LOEWY, L. WILLARD FREEMAN, ALBINO MARCHELLO AND VICTOR JOHNSON

From The University of Chicago

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Lymph samples collected from the lacteals and thoracic ducts of dogs following fat ingestion and absorption are markedly hemolytic; over half of the erythrocytes in samples of heparinized blood may be destroyed by mixing the blood with fatty chyle (Johnson and Freeman, 1938). The hemolytic factor in chyle is probably soap plus free fatty acids, which are present in chyle during the period of rapid fat absorption in quantities (ranging from 3.3 to 6.3 mgm. per cubic centimeter) sufficient to account for the hemolytic action of chyle (Freeman and Johnson, 1940).

Since this hemolytic action is demonstrable in chyle-blood mixtures in vitro, the question arises whether hemolysis actually occurs in the blood stream following fat ingestion. The work reported here is an attempt to determine whether a high fat diet accelerates red blood cell destruction. Following fat ingestion and absorption, chyle reaches the blood stream by way of the thoracic duct and other routes (recently reinvestigated by Freeman, 1942), and the circulating blood is exposed to a substance which has been demonstrated to be extensively hemolytic in vitro. When red blood cells are destroyed in the blood stream, the porphyrin constituents of the split hemoglobin are quantitatively excreted in the bile of dogs as bilirubin (Broun, McMaster and Rous, 1923; Hawkins and Johnson, 1939). Hence, the determination of the total bilirubin output provides a reliable quantitative measure of the rate of red blood cell destruction, even in the presence of normally functioning hematopoietic tissue.

METHODS. Internal bile-fistula dogs were prepared both in the manner described by Kapsinow, Engle and Harvey (1935) and by anastomosing the common bile duct with the right ureter (after removing the gall bladder and the right kidney), so that the total bile output was diverted to the urinary bladder and excreted with the urine. The dogs were placed in metabolism cages and daily collections of the 24-hour urine-bile mixtures were begun when the animals were in good clinical condition and gaining weight.

The daily 24-hour samples were filtered through gauze and the volume measured. Bilirubin determinations were made daily by a modification of the oxidative process of Malloy and Evelyn (1937). An aliquot portion of the urine-bile mixture was diluted with ethyl alcohol (95 per cent) to which was added the oxidizing agent, consisting of hydrogen peroxide in acid alcohol (0.5 cc. of 30 per cent H_2O_2 in 94.5 cc. of 95 per cent ethyl alcohol plus 5.0 cc. of concentrated HCl). The aliquot was usually 2.0 cc., since this quantity most often resulted

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

in a reading which fell in the center of the galvanometric scale of the Evelyn photoelectric colorimeter. When samples were highly concentrated, accurate dilutions were made to give readings in the proper range. Readings were made on all samples every one-half hour until a maximum blue color was developed. By this method quantitative recovery of known amounts of bilirubin (Eastman Kodak Company) added to urine was possible with an error not in excess of 2.0 per cent.

In addition, external bile-fistula dogs were prepared and bile was collected under constant negative pressure in the manner described by Kocour and Ivy (1938). Bile samples were collected every 24 hours and bilirubin determinations were carried out (using 0.5 cc. aliquot portions) as described above.

All dogs were maintained on a basal diet consisting of canned commercial dog food supplemented daily with cod liver oil (4.0 cc.), yeast (0.5 gram), iron (ferrous ammonium citrate, 100 mgm.), and bone meal (sufficient to control stool consistency). Each dog also received a daily dose of 0.5 gram of pure sodium taurocholate per os before feedings. Water was given ad libitum. To this basal diet was added olive oil or corn oil in amounts of from 5.0 to 10.0 cc. per kilogram of body weight to comprise the "high fat" diet. Cane sugar was substituted for the olive or corn oil in calorically equivalent amounts to comprise the "high carbohydrate" diet. Observations were continued for several months on most dogs. For each animal the diets to be compared were alternated several times. Each dietary regimen was usually maintained for a period of eight days to three weeks.

Results. The results of adding fat to the basal diet are shown graphically in figure 1. The average daily total output of bile pigment in milligrams is plotted against the number of days of observation. The figure indicates that at each shift from the control basal diet (cross-hatched) to the high fat diet (solid) the bile pigment output increases. With each change from high fat to control, the output decreases. The daily fluctuations in bile pigment excretion (not shown in fig. 1) are considerable. However, statistical analysis of the data on daily excretions for the 133 days of the experiment shows the bile pigment output to be significantly higher on the high fat diet.

To determine whether this increase might be caused by the increased caloric intake on the high fat diet, observations were made comparing bile pigment excretion on the high fat diet and on a diet in which this fat was replaced by calorically equivalent quantities of cane sugar. The results are plotted in figure 2, which shows that bile pigment excretion is greater on a high fat diet than on a calorically equivalent high carbohydrate diet. Statistical analysis of the figures on daily excretion for the 144 days of the experiment shows the differences to be significant. Hence, it appears that the increase in bile pigment output which occurs when dogs are placed on a high fat diet is due not to an increased caloric intake, but to increased red blood cell destruction produced by the products of fat digestion.

The composite results of observations on seven dogs are shown in figure 3. Here the average daily output of bile pigment for all dogs during all control

periods (cross-hatched column) is arbitrarily placed at 100 per cent. Each of the 24 points in the control column represents the average daily excretion of about 21 days of observations, totalling 511 dog-days. The average output during

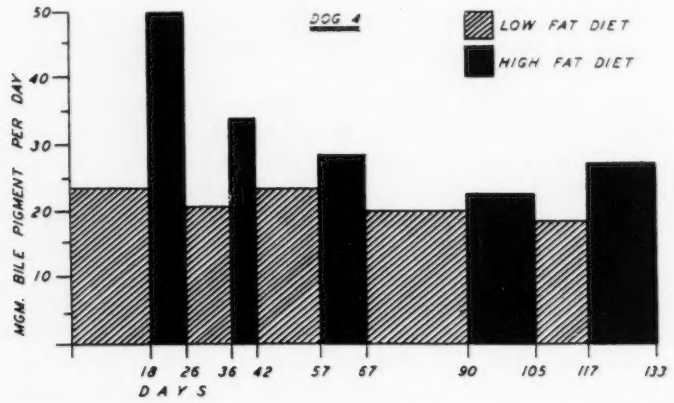

Fig. 1. Average daily excretion of bile pigment by a dog on the basal diet ("low fat"), and on the basal diet plus fat ("high fat").

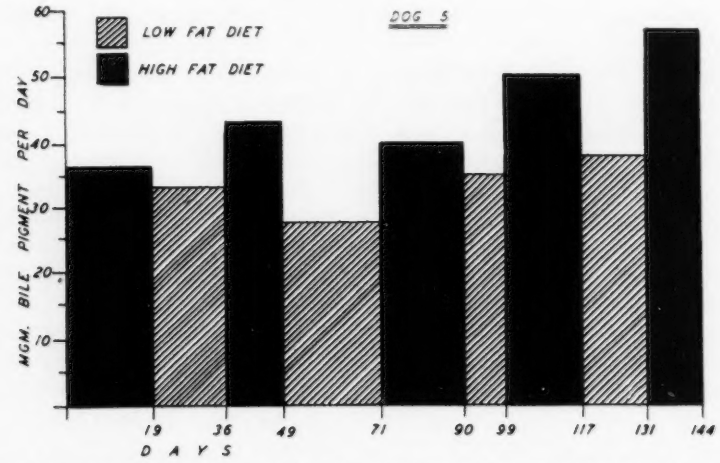

Fig. 2. Average daily excretion of bile pigment by a dog on a high fat diet, and on a low fat diet in which the fat was replaced with a calorically equivalent quantity of carbohydrate.

the administration of the high fat diet (solid column) is 137 per cent. Each of the 20 points in this column represents the average of about 16 days, the total being 325 dog-days. Statistical analysis reveals that the difference is significant despite the fact that two dogs responded poorly to the changes in diet.

Similar results were obtained following dietary changes in two external bile-fistula dogs.

Routine red blood cell counts made at weekly intervals in all of the experimental animals varied from week to week, but did not correlate with fluctuations in bilirubin output. No significant anemia was detected in any of the animals at any time.

Discussion. It has been postulated that red blood cells are destroyed in the body by phagocytosis, hemolysis and fragmentation. Rous (1923), in reviewing the literature, concludes that there is no positive evidence supporting any of these mechanisms or any combination of them. Isaacs (1937) points out that there is little evidence for phagocytic destruction. He states that a hemolytic mechanism is logical but has not been demonstrated. Fragmentation of red blood cells has been observed in peripheral blood in erythroblastic anemia

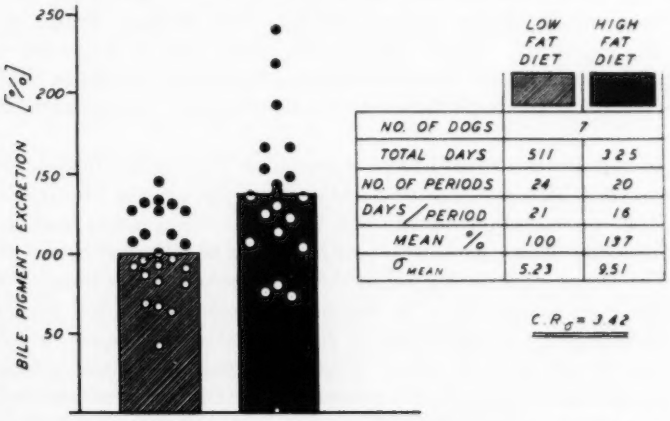

Fig. 3. Summary of results on bile pigment excretion in seven dogs on high fat and low fat diets.

(Cooley and Lee, 1933), and in other pathological conditions (Doan and Sabin, 1926). From the present work it would appear that the red cell destruction by chyle is probably by hemolysis, and that this mechanism may play a rôle in the normal red blood cell destruction in dogs. Further support is lent this concept by the experiments of Longini, Freeman and Johnson (1942), demonstrating that red blood cells exposed to lipemic serum are more fragile than cells exposed to fasting serum. Less direct evidence of red blood cell destruction from fat ingestion, based on studies of urobilin excretion, has been presented in a review by Glanzman (1929) and in a preliminary report of experiments by Josephs, Holt, Tidwell, and Kajdi (1938).

Hawkins and Whipple (1938) estimated the average life of the red blood cell at 124 days in dogs by determination of the time interval between the immediate rise in bile pigment output following massive destruction of circulating red cell and a later massive rise in bile pigment output. Calculating the average life of

red blood cells from the average daily output of bilirubin in the present studies yields the figure of about 200 days.

One might expect that, since a high fat diet causes increased red blood cell destruction in dogs, prolonged fat feeding would result in an anemia. In the present series of animals the number of erythrocytes remained essentially constant even though they were destroyed at an increased rate. The normally functioning hematopoietic tissue seemed able to compensate for the extra losses induced by fat feeding.

A blood picture simulating pernicious anemia has been reported by Crandall, Finne and Smith (1941) in bile fistula dogs somewhat like our own. However, this finding could scarcely be attributed to fat ingestion, since bile salts were withheld from the diet of their dogs, and presumably fat absorption was minimal.

Sodium taurocholate (Merck-"Pure") was fed our dogs in amounts calculated to substitute for the normal daily secretion. Pure bile salts have been shown not to influence bile pigment output (Hooper, 1916; Berman, Snapp, Atkinson, Ivy and Hough, 1940). Whole bile was fed at no time, as it was felt that absorption and re-excretion of bilirubin (Broun, McMaster and Rous, 1923) might affect the results. Although the stools were consistently clay-colored, they were not fatty.

Whipple and Hooper (1916) have reported an increased bilirubin output in dogs placed on a high carbohydrate diet. Rous, Broun and McMaster (1923) reported that no variation occurred in 24-hour samples from feeding a high carbohydrate diet except for a transient hastening of bilirubin output for a few hours. The latter workers suggested that the six-hour collections of Whipple and Hooper were inadequate. In the present series it was felt that possible caloric effects could be eliminated by feeding calorically equal carbohydrate control and experimental fat diets. Feeding periods were usually maintained for eight or more days, since it was observed in preliminary experiments that the response to a change of diet failed to occur, in some instances, until two or three days had elapsed.

Other factors which have been demonstrated to influence bilirubin output in bile-fistula dogs are operative trauma, exercise, infection, fever, and intercurrent diseases (McMaster, Broun and Rous, 1923; Broun, 1923). These factors were adequately controlled in the present work. After returning to good clinical condition the animals showed no evidence of fever or infection, daily exercise was only moderate, and body weight was maintained or increased. One dog died after 24 months; five were sacrificed after 21 months. Autopsies revealed only a left kidney infarct in one animal.

SUMMARY AND CONCLUSIONS

1. In five internal bile-fistula dogs the daily total bilirubin output was significantly higher on high fat diets than on calorically equivalent low fat diets.

2. This effect was also demonstrated in two external bile-fistula dogs.

3. These findings indicate that red blood cell destruction proceeds at a faster rate on a high fat diet than on a low fat diet; this effect is probably caused by the

introduction of a hemolytic substance into the blood stream by way of the lymphatics.

4. These sequelae of the ingestion of fat are probably one mechanism for normal red blood cell destruction in dogs.

5. Calculations from the average daily bilirubin output in this series indicate that the average life of the red blood cell in dogs approaches 200 days.

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THE SECRETION OF ALKALINE PHOSPHATASE BY THE DOG'S INTESTINE

A. J. KOSMAN, J. W. KAULBERSZ AND SMITH FREEMAN

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

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The presence of alkaline phosphatase in the small intestine has been recognized for many years. Grosser and Husler (1) and Levene and Medigreceau (2) were among the first to determine the activity of this enzyme in the intestinal mucosa, and since then extracts of the mucosa have been used as a potent source of the enzyme. In his review, Kay (3) points out that the mucosa of the small intestine had the highest phosphatase activity of all the animal tissues studied.

However, little attention has been paid to the phosphatase activity of the intestinal secretions. Levine and Dillon (4) prepared a nucleotidase from the secretions collected from an intestinal fistula in the dog. In his monograph, Robison (5) states that phosphatase is secreted by the small intestine without adducing any experimental evidence for its secretion, assuming perhaps that since it is present in such high concentrations in the mucosa it would appear in the succus entericus. In a recent review of the intestinal secretions, Florey et al. (6) refer briefly to Levene's nucleotidase but do not classify phosphatase as an enzyme regularly present in the intestinal juices.

The present investigations were undertaken in order to determine if phosphatase is a constant and active component of the intestinal secretions and to ascertain any factors that might influence its activity.

MATERIALS AND METHODS. Two groups of experiments were carried out. In one group an enterostomy was performed on dogs at three levels: the upper jejunum, the lower jejunum and the lower ileum. A small rubber tube, through which samples of the intestinal contents could be collected, was inserted at each of these levels and exteriorized through a stab wound. Four dogs were prepared in this manner and the effects of various drugs and diets on the phosphatase activity of their intestinal secretions were determined.

The animals were fasted for twenty-four hours and then were fed a test meal either ad libitum or by stomach tube, depending upon the nature of the meal. Two hours after feeding the animals were placed in stocks and samples of their chyme were collected for a period of two hours. Immediately upon collection the samplings were centrifuged for a half-hour at 2200 r.p.m. The phosphatase activity and inorganic phosphorus of the supernatant portion were determined. Phosphatase activity was measured by the method of Bodansky (7) and inorganic phosphorus by the method of Kutner and Lichtenstein as modified by Bodansky (8). The test meals were meat, milk, casein, olive oil, corn oil, and sucrose.

Another series of experiments was carried out in which the animals were injected with 3 to 5 mgm. of eserine subcutaneously following the period of fasting.

Collections of the intestinal contents and phosphatase and inorganic phosphorus determinations were carried out in the manner outlined above.

In a second group of experiments, isolated loops, 12 inches long, of the duodenum, jejunum and ileum were prepared in dogs under nembutal anesthesia. The loops were thoroughly washed with saline, after which the proximal end was closed off and a cannula inserted in the distal end. The animals were injected with 3 to 5 mgm. of eserine subcutaneously and the secretions of each loop collected hourly for a period of three hours. The collections were centrifuged immediately and phosphatase and inorganic phosphorus determined in the manner described above.

Some observations were also made on the secretions collected from jejunal and colonic fistulae of unanesthetized dogs.

RESULTS. *The influence of diet on the phosphatase activity of intestinal chyme.* A high phosphatase activity was found in the intestinal samplings following all

TABLE 1

DIET	DUODENUM		JEJUNUM		ILEUM	
	Phosphatase*	Inorganic P†	Phosphatase*	Inorganic P†	Phosphatase*	Inorganic P†
Casein (7)	2170	62.9	4404	78.0	23989	107.3
Olive oil (10)	2475	6.5	6830	4.2	19799	3.4
Corn oil (3)	2030		10512		21813	
Meat (11)	1034	89.5	4278	55.9	11862	18.7
Milk (8)	725	54.4	2966	32.4	5168	12.6
Sucrose (8)	842	9.6	1058	4.8	6358	7.2
Eserine (5)	2166	4.3	5168	8.7	9930	10.0

Bracketed numbers indicate the number of experiments.
* Bodansky units per 100 cc. of intestinal contents (average).
† Milligrams per 100 cc. of intestinal contents (average).

the test meals and eserine injection. These results are shown in table 1. The greatest activity at each of the three levels of the intestine was obtained after the casein and oil feedings. The average values in Bodansky units after casein were 2170, 4404, and 23989 and after oil were 2252, 8671, and 20806 at the duodenal, jejunal and ileal levels respectively. The activity of the chyme was about half as great following the meat meal and the least activity was obtained following the milk and sugar feedings.

There is an increase in the phosphatase activity of the intestinal contents as they pass caudalward, the values of the ileal chyme being 7 to 10 times greater than those of the duodenum.

The secretion of phosphatase by isolated intestinal loops. The phosphatase activity of the secretions collected from the duodenal, jejunal and ileal loops is shown in table 2. The duodenal and jejunal secretions exhibited the greatest activity, 2491 and 2048 Bodansky units, respectively. The phosphatase values of the ileal secretions were definitely lower, 429 units. The activity gradient

of the isolated loops is thus the reverse of the gradient found in the experiments on the enterostomized dogs described above. This would indicate that the increased activity of the ileal chyme collected from the enterostomized dogs is probably due to concentration of the intestinal contents as they pass down the intestinal tract and does not represent a greater secretion of the enzyme by the lower part of the gut.

The activity of the hourly collections remained fairly constant over the total collection period of three hours. The volume of the secretions from the duodenum and jejunum averaged 15 to 20 cc. an hour and the ileal flow, 3 to 5 cc. an hour.

A high phosphatase activity was also found in the secretions obtained from dogs with chronic jejunal Thiry-Vella fistulae. These values agree with those found in the jejunal secretions of the acute dogs. The secretions of the chronic colon fistulae exhibited the least activity, 250 Bodansky units per 100 cc. of secretion. These results are shown in table 2.

TABLE 2

LOCATION OF LOOP	PHOSPHATASE*	INORGANIC P†
Duodenum (12)	2491	5.3
Jejunum (12)	2048	3.8
Ileum (12)	429	2.9
Chronic Thiry-Vella loop of jejunum (14)	2118	3.3
Chronic Thiry-Vella loop of colon (7)	252	1.2

Bracketed numbers indicate number of experiment.
* Bodansky units per 100 cc. secretion (average).
† Milligrams per 100 cc. secretion (average).

DISCUSSION. The observations on the enterostomized dogs indicate that there is a high phosphatase activity of the intestinal contents at all times. Since the samplings represented the mixed secretions of the stomach, small intestine, pancreas, and bile, the phosphatase activity of each of these secretions must be considered in determining the major source of the enzyme in the intestinal fluids. The phosphatase activity of gastric and pancreatic juice is negligible. Bile, however, does contain considerable quantities of the enzyme. According to Freeman and Ivy (9) fistula bile of the dog has an activity of 35 to 118 Bodansky units per 100 cc. and gall bladder bile may have values several times higher. Armstrong et al. (10) found higher values for liver and bladder bile, using a different substrate and unit of activity, however. These latter workers have also shown that the fecal phosphatase of bile fistula and obstructed bile duct dogs remains at its typically high level despite the exclusion of bile from the intestinal tract.

Since the phosphatase activity of the intestinal contents at the duodenal level is greater than the reported values for bile and since the bile must undergo dilution by the other secretions and the ingested food, it can be assumed that the

greater part of the phosphatase activity of the chyme is due to the secretions of the intestinal mucosa. Similarly, the great activity of the enzyme in feces most probably has its source in the intestinal secretions.

The high phosphatase activity of the uncontaminated secretions of the isolated intestinal loops in both the acute and chronic dogs lends support to these assumptions.

Practically all collections of succus entericus are contaminated by cellular debris and this fact has led some investigators (Florey et al., 6) to question the reported presence of various enzymes in the intestinal juices as due to a true secretory activity of the mucosa. Whatever the merits of such a criticism may be, the great phosphatase activities found in all the samples of the intestinal secretions, collected under various conditions and subjected to an immediate and vigorous centrifuging, could hardly be accounted for by cell contamination alone. Nor did the activity of samples before and after centrifuging show any change in several instances in which such estimations were carried out. The experimental evidence presented indicates that alkaline phosphatase is secreted by the intestinal tract in varying but considerable quantities, the duodenum and jejunum being the major sites of its secretion.

The significance of the increased enzyme activity of the chyme following the high fat and protein meals is not clear. The question of the adaptive secretion of the small intestine is a confused and controversial one. Bourne et al. (11) have shown that the secretions of an isolated intestinal loop in the dog are richest in enzymes following high fat and protein diets and least after carbohydrate, although there was no change in the relative concentrations of the enzymes studied. Since in the present investigations no observations were made on the activity of other enzymes, it cannot be stated whether the increased phosphatase activity after fat and protein feedings is a specific response or not. Cera and Bellini (12) have reported an increase in the phosphatase activity of the intestinal mucosa of the albino rat during fat absorption. Westenbrink (13), however, found no discernible relationship between the character of the diet and the phosphatase activity of intestinal mucosa in the rat.

The choleretic action of protein and fat and the cholagogic action of fat may also contribute to the increased activity following these meals by augmenting the amount of bile in the intestinal tract.

The function of alkaline phosphatase in the intestinal tract still remains to be ascertained although the wide occurrence of monophosphoric esters in food would suggest a plausible rôle for the enzyme in the digestive process.

SUMMARY

1. The phosphatase activity of duodenal, jejunal and ileal contents obtained from enterostomized dogs was determined following various test meals.

2. The greatest activity was found after protein and fat meals and the least following carbohydrate.

3. The secretions of isolated intestinal loops of dogs with acute or chronic

Thiry-Vella fistulae contain large quantities of phosphatase. The degrees of activity of various segments of the intestinal tract are as follows: duodenum and jejunum > ileum > colon.

4. Phosphatase is considered to be a true secretion of the intestinal tract; its function and the effect of diet upon its activity are discussed.

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INFLUENCE OF NEPHRECTOMY ON OVARIAN RESPONSE TO GONADOTROPINS

FRITZ BISCHOFF AND GEORGENA J. CLARKE

From the Chemical Laboratory, Santa Barbara Cottage Hospital Research Institute, Santa Barbara, California

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It is possible, since attempts to demonstrate differences in chemical structure for the various gonadotropins have been unsuccessful (1), that the prosthetic groups[1] of all the gonadotropins partake of the same general structure, any difference in physiologic response being the result of differences in physical properties of the molecule or micelle as a whole, which might affect the rate of resorption, permeability, destruction, excretion or stimulation to anti-body formation. The importance of the rate of resorption has been established by investigation on divided dosage (2), on administration of insoluble combinations (3), and on retarding or accelerating body fluid exchange (4). It is the purpose of this paper to extend the study to the kidney to determine whether or not this organ is involved. To this end a comparison has been made between intact and partially nephrectomized rats of the action of three characteristic gonadotropins from sheep pituitary, pregnant-mare serum, and urine of human pregnancy respectively.

Parkes and White showed that 30 per cent of injected prolan appeared in the urine of the rabbit within 9 hours (5). This observation was confirmed by Lipschütz (6), who found in addition that prolan disappeared less rapidly from the blood of the nephrectomized rabbit than it did in the normal rabbit. Evans, Simpson and Austin (7) found prolan in the urine of the rat injected with this substance. These workers and Catchpole, Cole and Pearson (8) were unable to recover mare-serum hormone from the urine of injected animals. Data on the excretion of pituitary hormone by the rat kidney are not available, so that an experiment to cover this point was included.

EXPERIMENTAL PROCEDURE AND RESULTS. *Excretion of sheep pituitary hormone by the normal rat.* The urethras of five 200 to 300 gram female rats were tied off under ether anesthesia. Eight to 11 mgm. of sheep pituitary gonadotropin in saline solution was injected subcutaneously. The bladder contents were collected 6 hours later. The urine was treated with an excess of tannic acid. The precipitate which formed was removed by centrifugation and taken up in a volume of isotonic saline solution corresponding to the volume of urine used. The tannic acid suspension was injected into 22-day-old female rats in 4 divisions once daily in an amount which would correspond to 1.7 to 3.0 mgm. of original hormone if all appeared in the urine unchanged. Tannate suspensions

[1] It is of course also possible that prosthetic groups, in terms of specific atomic linkages are non-existant in the protein molecule, physiologic activity depending upon specific conditions for adsorption (secondary valence effects).

of the original hormone were administered to 22-day-old female rats at 3 dosage levels.

	DOSE PER RAT	AVERAGE WT. OF OVARIES	AVERAGE WT. OF UTERUS	NO. OF RATS
	mgm.	*mgm.*	*mgm.*	
Original hormone	1.0	63		6
	0.5	34	89	6
	0.2	12	73	6
	0.0	14	17	4
Urine precipitate equivalent to original hormone if all were excreted	1.8	14	29	4
	1.7	14	15	3
	3.0	14	15	3

The experiments establish that less than 10 per cent of the hormone was present in the urine in unchanged form 6 hours after administration.

Administration of three gonadotropins to nephrectomized rats. Twenty-two-day-old female rats were partially nephrectomized by the technic of Chanutin and Ferris (9). The first dose of hormone was administered within the ensuing 24 hours. In the case of the mare-serum hormone (Cutter's gonadin), 10 Cole units were administered per rat in a single injection. The sheep pituitary gonadotropin and the human pregnancy prolan, which were the stable powders previously used in this laboratory (10, 11), were administered in isotonic saline solution, 1 dose per day on 4 consecutive days. The animals were autopsied 96 hours after the initial dose. In addition to ascertaining organ weights and body weight at autopsy, blood pressure readings by the method of Williams, Harrison and Grollman (12), blood non-protein nitrogen, and dry weight of the hearts, were determined in certain instances. In each experiment a comparison was made between litter mates, there being 6 or 7 pairs used for compilation in each experiment. A certain number of the operated rats succumbed during the period of dosage. The corresponding litter mates were then removed from the experiment. Rats which weighed less than 35 grams at the end of the experiment, with their corresponding litter mates, were eliminated from the experiment.

The choice of the level of hormone administered was made by selecting that level which would produce ovarian hypertrophy in the range of greatest sensitivity to dose increment, when administered under optimum conditions as far as they were known. Because of the nature of the results obtained with prolan, the experiment was repeated with double and eight time the dosage level. The conditions chosen for administration of the hormones were those used in earlier experiments (3, 4) so that a basis of comparison with these experiments is afforded.

Condition of nephrectomized rat. It will be noted that while the body weight of the nephrectomized rat is 81 to 84 per cent that of the intact rat, the heart weights are not proportionally decreased. Determination of the dry weight of 6 control hearts and 6 hearts of operated rats gave values of 21.5 ± 0.15 and

21.9±0.19 mgm., respectively. The increase in heart weight:body weight ratio in the nephrectomized rats is indicative of a true cardiac hypertrophy. The increase in blood pressure, which is doubtfully significant, is in line with the observations of Chanutin and Ferris. The consistent rise in the non-protein nitrogen of the blood establishes the condition of renal impairment. Kidney hypertrophy even in the 4-day period is noted, though this may be due in part to hydration.

Ovarian and adrenal weight changes. Since the body weight of the nephrectomized rats was 81 to 84 per cent of that of the intact rats at the time of autopsy, the nephrectomized rats received relatively more hormone on a weight

TABLE I

Influence of nephrectomy upon the response to the gonadotropin in immature female rats

BLOOD PRESSURE	BLOOD NPN MEAN*	TREATMENT	GONADOTROPIN AND DOSE TOTAL PER RAT	BODY WT. MEAN	OVARIAN WT. MEAN	KIDNEY WT. MEAN	HEART WT. MEAN*	ADRENAL WT. MEAN	UTERINE WT. MEAN
mm. Hg	*mgm %*			*gm.*	*mgm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*
	47 (4)	Control intact		51 ±1.0	16 ±1.0	0.79 ±.02	245 (4)	18 ±2.6	16 ±0.9
	46 (4)	Intact	Pituitary 1 mgm.	49 ±1.6	17 ±0.9	0.79 ±.02	230 (4)	19 ±1.1	25 ±4.5
	71 (4)	Nephrectomized		43 ±1.8	14 ±1.2	0.37 ±.01	240 (4)	17 ±0.7	16 ±1.2
	79 (4)	Nephrectomized	Pituitary 1 mgm.	41 ±1.2	25 ±3.1	0.39 ±.02	243 (4)	19 ±0.9	37 ±4.1
87 ±3	34 (5)	Intact	Mare serum 10 u.	47 ±2.0	53 ±4.4	0.72 ±.03	258 ± 8	16 ±0.6	
99 ±4	60 (5)	Nephrectomized	Mare serum 10 u.	38 ±2.4	73 ±8.3	0.30 ±.01	269 ±19	25 ±2.2	
	44 (5)	Intact	Prolan 0.3 mgm.	52 ±2.2	32 ±2.7	0.82 ±.07	258 ±10	17 ±0.4	
	76 (5)	Nephrectomized	Prolan 0.3 mgm.	43 ±2.1	60 ±5.4	0.34 ±.03	255 ± 9	17 ±1.0	
		Intact	Prolan 0.6 mgm.	53 ±2.7	41 ±3.4	0.81 ±.03	273 ±17		
		Nephrectomized	Prolan 0.6 mgm.	41 ±2.5	76 ±8.7	0.29 ±.02	230 ±13		
		Intact	Prolan 2.4 mgm.	50 ±1.2	46 ±2.2	0.72 ±.02	247 ±10	18 ±0.5	
		Nephrectomized	Prolan 2.4 mgm.	40 ±0.7	76 ±9.3	0.23 ±.01	235 ± 9	23 ±1.6	

* The figures in parentheses indicate the number of animals used to obtain the particular data recorded. When not stated the number is 6 or 7. The statistic is the standard deviation of the mean.

basis. A 20 per cent increase in mare serum administration to intact rats over the 10 units administered would produce a 20 mgm. increase in ovarian weight (13). The result obtained in the nephrectomized rats which received mare serum hormone could, therefore, be accounted for by a higher level of hormone on body weight basis. Under the conditions of dosage in the prolan experiment, a 20 per cent increase of prolan dosage to the intact rats would only produce a 2 to 3 mgm. increase in ovarian weight (1). The 28 mgm. increase on the low dose and the 35 mgm. increase on doubling the dose obtained in the operated rats is, therefore, marked and highly significant. However, on increasing the dose still further (eightfold the smaller dose), no further augmentation was noted above the 35 mgm. increase. Even a schedule of divided dosage employing 5 instead of 1 dose per day increases the ovarian response to prolan in intact rats

by only 12 mgm. (2). Administration of the insoluble copper compound produced an increase of 15 to 20 mgm. The pituitary hormone given in these experiments in soluble form once per day and at a 1 mgm. level (total) produced no significant increase in ovarian weight in intact rats and an 11 mgm. increase in nephrectomized rats. Five divided doses per day of the same total dose of hormone, however, produce 50 mgm. ovaries in intact rats (14), administration as the insoluble copper combination produces 100 mgm. ovaries in intact rats, and administration in soluble form to thyroidectomized (4) animals produces 28 mgm. ovaries. It requires 4 mgm. total dose administered once per day for 4 days to produce 28 mgm. ovaries (14). Nephrectomy and thyroidectomy effect the response to the pituitary hormone to approximately the same degree.

There is no indication that nephrectomy influenced the action of endogenous hormone.[2] The data in the table for ovarian and uterine weights for the non-treated nephrectomized rats are within the range of the control data. It is, however, true that the amount of pituitary hormone which will produce specific augmentation of prolan is barely detectable in intact rats under the conditions of our experiment (2). The largest ovarian weight produced by prolan in any of the intact rats was 62 mgm. In 3 nephrectomized rats, ovarian weights over 110 mgm. were produced through administration of prolan. It will be noted that the absolute increase in ovarian weight in the nephrectomized rats, which received prolan, above that produced in the treated intact rats averaged approximately 30 mgm. at each dose-level covering an eightfold dosage range.

Adrenal weights were not significantly changed by nephrectomy or by administration of any of the gonadotropins to intact animals; they were, however, significantly raised in nephrectomized rats by administration of mare-serum hormone and by the large dose of prolan, but not by the small dose of prolan nor by the pituitary hormone. The significance of this finding is not understood.

SUMMARY

Twenty-two-day-old rats were partially nephrectomized by the technic of Chanutin and Ferris. Four days later body weight was approximately 20 per cent below that of litter-mate controls, heart weight was equal to that of controls, the blood non-protein nitrogen was elevated 30 mgm. per 100 cc., and the blood pressure was slightly elevated. Solutions of sheep pituitary gonadotropin, of prolan, and of purified pregnant mare-serum hormone were administered to operated and to litter-mate intact rats.

[2] It is well known that whereas the mare serum and pituitary gonadotropins produce ovarian hypertrophy in the hypophysectomized rat, prolan has a greatly diminished effect. It has been assumed that the action of prolan in the intact rat is due to a synergism produced by the presence of endogenous hormone. In the present experiments an attempt was made to study the action of prolan in the hypophysectomized partially nephrectomized rat. It was found that when the two operative procedures were performed within 48 hours the mortality was high or the surviving animal lost so in body weight that results would have little significance. The results, nevertheless, suggest the possibility that a lowered renal threshold in the hypophysectomized rat may be a factor in the lack of ovarian response.

In the partially nephrectomized rats, the augmented response to pituitary hormone was of the same order as that produced by thyroidectomy, and much less than that produced in intact rats by divided dosage or administration of an insoluble compound. The augmented response to mare-serum hormone could be accounted for by body-weight difference (similar to the influence of thyroidectomy). The augmented response to prolan, which was 175 to 240 per cent, was greater than that produced by divided dosage, thyroidectomy, or administration of an insoluble form. Prolan was able to produce ovaries weighing more than 100 mgm. at the age of 26 days in partially nephrectomized rats.

The well known failure of prolan, in contrast to sheep pituitary and mare-serum hormones, to produce increases in ovarian weight of intact rats proportional to increases in level of hormone administered is interpreted as due to a low renal threshold for prolan.

Less than 90 per cent of injected sheep gonadotropin was recovered in the urine of the mature rat during a six-hour period.

Hypertrophy of the adrenals in nephrectomized but not in intact rats was produced following gonadotropin administration under certain conditions.

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HISTOLOGICAL EFFECTS IN RATS RESULTING FROM ADDING RUBIDIUM OR CESIUM TO A DIET DEFICIENT IN POTASSIUM

RICHARD H. FOLLIS, JR.

From The Department of Pathology, The Johns Hopkins Medical School, Baltimore, Maryland

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There is a good deal of evidence that Rb and to a lesser extent Cs can replace K in certain physiological processes. Ringer (1) noted that Rb corresponded to K in its action on the frog's heart, while Cs differed in that it resembled more closely the effect of Ba. Clark (2) showed that Rb may be substituted in place of K for the physiological activity of certain isolated organs; Cs was found to be an imperfect substitute. Loeb (3) reported that both Rb and Cs would replace K in solutions required for the development of sea urchin eggs into swimming blastulae. Using rats placed on a synthetic K low diet to which Rb or Cs had been added, Mitchell et al. (4) found that after several weeks the animals became hyperirritable and died shortly afterwards in violent tetanic spasms. Similar changes were noted even when K was added to the diets containing Rb or Cs. Chemical analyses revealed significant amounts of Rb and Cs in the heart and skeletal musculature, although no control studies were made. Heppel and Schmidt (5) have partially confirmed these observations of Mitchell et al. (4) and have concluded that "for a certain time in the growth period of the rat there is some physiological replacement of K by Rb." *In vitro* experiments by Mann et al. (6) have shown that Rb and to a less extent Cs acted as K in bringing about an increased formation of acetylcholine by respiring brain tissue.

Physiological (7) as well as anatomical changes (8, 9) have been observed in animals on diets extremely low in K. Necroses of both myocardial fibres and renal tubular epithelial cells have been noted in several species. The present experiments were designed to find out whether or not the anatomical integrity of the myocardium and kidney could be maintained when Rb or Cs were substituted for K in a diet extremely deficient in the latter element.

EXPERIMENTAL. The basal K-deficient diet used in this study was reported by Orent-Keiles and McCollum (10). It consists of lactalbumin, 10.0; wheat gluten, 4.0; gelatin, 4.0; sweet butter fat, 8.0; K-free salt mixture, 4.6; choline hydrochloride, 0.02, and sucrose (Dyno) to make 100 grams. Fifteen drops of cod liver oil are added to each kilogram of diet. Instead of using liver concentrate as the source of the B group, crystalline vitamins[1] were administered orally twice a week. Each animal received the following amounts per week: thiamine chloride, 0.2 mgm.; riboflavin, 0.28 mgm.; calcium pantothenate, 1.0 mgm.; pyridoxine, 0.2 mgm., and nicotinic acid, 1.0 mgm. Alpha tocopherol, 1.0 mgm. per animal, was given weekly. The substitution of the crystalline vitamins for

[1] Furnished through the courtesy of Dr. D. F. Robertson, Merck and Company.

liver concentrate produced a much more acute deficiency than we have observed heretofore.

Albino rats, weighing 35 to 50 grams, were used. The following are the modifications of the diet made by adding the appropriate salts to the basal ration, together with the number of animals placed on each diet: low K-group, basal diet alone, 6 animals; control group, basal diet plus 0.85 per cent KCl, 5 animals; Rb-substituted group, basal diet plus 0.5 per cent RbCl, 9 animals; Rb-control group, basal diet plus 0.5 per cent RbCl and 0.85 per cent KCl, 3 animals; Cs-substituted group, basal diet plus 0.5 per cent CsCl, 9 animals; Cs-control group, basal diet plus 0.5 per cent CsCl and 0.85 per cent KCl, 3 animals. The basal ration contains about 0.001 per cent K and the control diet contains about 0.44 per cent K. The following percentages of Rb or Cs were used respectively, 0.35 per cent and 0.39 per cent.

The animals were placed in screen bottom cages. Food and distilled water were given *ad libitum*. Microscopic sections of the heart, striated muscle and kidneys were made in all the animals. In addition, sections of all the tissues were made in several representative animals from each group.

RESULTS. *K-deficient group.* The animals on the basal diet (0.001 per cent K) grew poorly and died within 4 weeks (average 25 days). All showed necrotic foci in the myocardium and changes in the renal tubular epithelium similar to those we (9) have described elsewhere. The other tissues appeared to be normal.

Control group. The animals whose diet was supplemented with K (0.44 per cent) grew well. No changes were found in the heart or kidneys.

Rb-substituted group. In these animals growth was far better than in the K-deficient group but poorer than the controls. After being on the diet for about 10 days they became hyper-irritable. When touched they jumped away and cried. At this time it was found that auditory stimuli (rattling of keys, compressed air blast) produced a characteristic and uniform response. The animal first began to tremble, then ran wildly about the cage for 5 to 10 seconds. It then fell on its side with fore and hind legs extended and paws clenched. Though the extremities were spastic, there were fine muscular tremors. In a minute or so these became coarser and gave way to a disappearance of the spasticity while the rat lay seemingly exhausted. The entire episode lasted 3 or 4 minutes. The animals did not die during the first attack but survived a number, one as many as 7 on successive days. The average survival time was 16 days. Those that were observed to succumb did so following an attack.

On microscopic examination no lesions were found in the myocardium of any of the animals. No changes were found in the kidneys of this group of rats, nor were there lesions in the other tissues including the brain.

Rb-control group. These animals failed to grow as well as the Rb-substituted group; their growth was better than the animals on the basal diet. The average survival time was 14 days. No fits such as were observed in the Rb-substituted group were observed in these animals.

Microscopically there were lesions in the myocardium in all 3 of the animals of this group. In 2 the changes were extensive while in the remaining rat the

changes were less severe. In the kidneys and other tissues no changes were observed.

Cs-substituted group. In these animals growth was poorer than in the Rb-supplemented rats. Two of the 9 exhibited fits similar to those of the Rb group. These 2 were among the longest lived animals. The average duration of life was 12 days.

Microscopically there were lesions in the myocardium of 6 of the 9 animals. The kidneys of 3 animals also showed changes similar to those encountered in K deficiency. Those animals dying earliest failed to show myocardial or renal damage.

Cs-control group. These animals grew very well for the first 3 weeks, a little better even than the controls. At this time their growth became stationary and in the last week they lost weight. Coincident with this growth disturbance the rats became hyper-irritable and during the last few days of life exhibited the

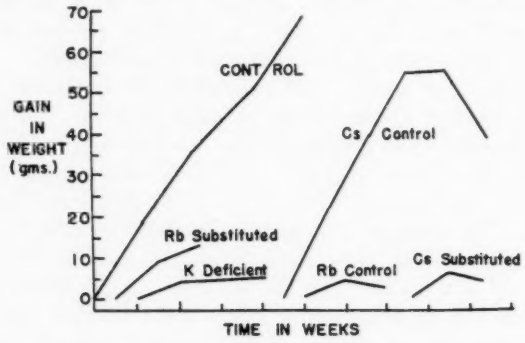

Fig. 1. Growth curves of various groups of animals

same fits that we had observed in the other animals. One striking thing about them was an extraordinary rigor present at death.

Microscopically no lesions were found in the heart and kidneys of these animals. The other tissues likewise showed nothing.

Discussion. When Rb was added to a K deficient diet, myocardial and renal lesions usually associated with K deficiency failed to appear. When Cs was added to the diet, renal changes were encountered in 3 of 9 and myocardial necroses were present in 6 of the 9 animals. Thus it would seem that during the period of observation, at least, Rb was able to replace K and so prevent the renal and myocardial changes, while Cs acted similarly but to a much less extent. The presence of lesions in the Rb control group raises the question as to whether this element is acting as a poison or whether it may be replacing K in the cell. Chemical determinations only can answer this question. In the case of Cs, lesions were not found in the animals dying earliest. It might be argued that there must be a certain time period before changes can take place and that their appearance might be modified by the rate of growth of the animals. On

this diet, however, we have evidence (13) that indicates that even when the food intake of the animals on the low K-diet is restricted so that they gain no weight, lesions appear after 11 days on the diet. Although we have no chemical proof that these elements had passed into the myocardial fibers or renal tubular epithelial cells, studies of Mitchell et al. (4) in the case of the heart, make this seem likely. It is interesting to point out that the main route of excretion of both these elements is via the kidneys (11, 12) and this may help explain the protective effect on this organ.

The seizures which were observed in all the animals on the Rb-substituted group, in a few of the Cs-substituted group, and in all the rats of the Cs-control group, are similar to those described by Mitchell et al. (4) and Heppel and Schmidt (5). Their cause is not clear; no histological changes in the nervous system were found. The fits seem to be identical to those described as specific in Mg deficiency (14), and following the deprivation of pyridoxine in rats (15).

From the effects on growth it seems that, in the proportions used in the diets, when K was present Rb acted as a poison while the reverse was true when K was absent. This observation is similar to what Mitchell et al. (4) found but contrary to that reported by Heppel and Schmidt (5), who observed good growth when K was added to the Rb-containing diet. It is not unlikely that the discrepancy is due to the different proportions of K and Rb in the respective diets. Our diet contained 0.44 per cent K and 0.35 per cent Rb. That of Mitchell et al. (4) was computed as containing 0.9 per cent Rb, the K content was not stated while the Heppel and Schmidt (5) diet contained 0.56 per cent K and 0.28 per cent Rb. The growth of the Cs-substituted group was poor while during the corresponding time the Cs-control animals exhibited vigorous growth for a time; with the onset of fits the growth curve began to fall.

The rôle, if any, which Rb and Cs may play in normal physiological processes is not at all clear; these elements are not considered to be indispensable ones. It should be pointed out, however, that Sheldon and Ramage (16) found Rb to be widely distributed in human tissues and to occur in highest concentration in the heart and skeletal musculature. Scott and Canaga (17) have found, of all tissues examined, Cs is present in the retina of oxen. The significance of this is obscure.

SUMMARY

Microscopic studies were made of the hearts and kidneys of rats that had been placed on a low K-diet to which either Rb or Cs had been added. Whereas myocardial and renal necroses appeared in control animals in the low K-diet, the addition of Rb prevented their appearance. Cs partially protected the kidney and to even a lesser extent the heart. Peculiar fits such as have been described by others were observed when Rb and Cs were added to the diet.

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THE INFLUENCE OF ATROPINE ON THE ATROPHY OF DENERVATED SKELETAL MUSCLE OF THE MONKEY (MACACUS RHESUS)

SAMUEL SOSKIN AND R. LEVINE

From the Department of Metabolism and Endocrinology,[1] Michael Reese Hospital, Chicago

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On the basis of a study of the dynamics of the chemical changes in denervated muscles of rats (1) we have previously postulated that the unceasing fibrillary activity of the muscle fibers may account for the atrophy which follows nerve section. There is reason to believe that the fibrillation is due to a sensitization of the denervated muscle to the minute amounts of acetylcholine normally present in the body fluids (2). We therefore administered atropine to rats on which nerve section had been performed, and showed that, under our experimental conditions, this drug was able to reduce the degree of atrophy by 39 per cent as compared to untreated control animals (3). The dose of atropine which was effective in our experiments with rats was high as judged by ordinary pharmacologic standards, but most of the animals maintained their body weights. However, the rat is notoriously resistant to atropine and there is little information available as to species difference in susceptibility to this drug. In the present work we tested the effect of atropine on the atrophy of denervation in monkeys, as a further step towards the possible therapeutic application of the drug in humans.

METHODS. Female monkeys (Macacus rhesus) ranging in weight from 3.0 to 4.3 kgm. were used throughout. One hind limb was denervated by section of both the sciatic and femoral nerves. The other hind limb served as the weight control. Twenty-one days after denervation[2] the gastrocnemii of both hind limbs were removed and weighed immediately. The difference in their weights represented the percentage atrophy suffered by the denervated muscle. In the treated monkeys atropine (sulphate) was administered subcutaneously, twice daily, for the 21-day period. The total dosage per animal per day was varied for different groups of animals, as detailed in table 1.

RESULTS. Table 1 summarizes our data. The average per cent atrophy in the untreated group was 32.5 per cent. Except for the smallest daily dose of atropine, one or more animals in all the treated groups responded with a significant diminution of the degree of atrophy. Regardless of the dose, all animals which did respond to atropine showed about the same inhibition of atrophy, ranging from 26 to 39 per cent. Those monkeys which did not give this response, gave practically no response at all. That is, an "all or none effect" is suggested. However, our data also suggest that with the highest doses used, more of the

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

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TABLE 1

Effect of varying doses of atropine upon the atrophy of denervated gastrocnemii in monkeys, 21 days after nerve section

NO. OF MONKEYS	ATROPINE	BODY WEIGHT		PER CENT ATROPHY OF GASTROCNEMIUS	PER CENT DEVIATION† FROM CONTROL	
		Initial	Final		Not significant	Significant
	*mgm./day**	*kgm.*	*kgm.*			
		3.5	4.0	33‡		
4	0	3.0	3.6	29‡		
	(Control)	3.8	4.0	33‡		
		4.0	4.2	35‡		
				32	−2	
2	0.5			28	−13	
		3.6	4.0	33	+2	
2	1.0	3.8	4.2	20		−39
2	2.0	4.0	4.0	35	+8	
		3.0	3.4	22		−32
		4.1	4.0	35	+8	
3	3.0	3.0	3.2	24		−26
		3.5	3.0	22		−32
		3.8	4.0	32	−2	
3	4.0	3.6	4.0	28	−13	
		3.7	3.9	23		−29
		3.4	3.8	32	−2	
3	5.0	4.0	3.6	32	−2	
		4.1	4.0	21		−38
		3.8	4.0	35	+8	
		4.3	4.2	31	−5	
		3.0	3.3	31	−5	
8	10.0	3.8	3.6	24		−26
		3.5	3.9	23		−29
		4.0	4.2	22		−32
		3.5	3.3	20		−39
		3.7	4.0	20		−39
		4.1	4.0	24		−26
3	15.0	3.6	3.8	24		−26
		3.8	3.9	20		−39

* The indicated daily dosage was given subcutaneously in 2 equal portions, and administered from the day after the nerves were sectioned until the day upon which the muscles were removed for examination.

† Plus sign indicates increased atrophy; minus sign indicates decreased atrophy.

‡ Average per cent control atrophy 32.5.

animals exhibited significant inhibition of atrophy. As regards the possible general effects of the atropine, only one animal out of the 30 lost a significant amount of weight during the treatment, and that animal was in the lower dosage range. With this exception all the monkeys exhibited only minor fluctuations in body weight, even at the highest dose levels.

Discussion. The present work confirms in the monkey our previous results and conclusions as to the effect of atropine on the atrophy of denervation in rat muscle (3). This effect is, therefore, not restricted to one species. Moreover, in the monkey, the effective doses of atropine are much smaller on the basis of body weight than in the rat. In rats we used 15 mgm. of atropine per 100 grams body weight, per day (given subcutaneously in two equal parts), while in the present work the highest dose averaged 0.5 mgm. per 100 grams body weight per day. With this dose, and to a lesser extent with the next highest dose, the monkeys were not as bright and active as usual, although no other manifestation of toxicity was evident. The fact that our treated animals maintained their body weights precludes the possibility that the inhibition of atrophy might have been due to a differential loss of weight by the normal and denervated muscles, because of undernutrition.

It is difficult to predict the doses of atropine which might prove effective in human beings. But, considering their bulk as compared to rats and monkeys, it seems quite possible that amounts of atropine which could be tolerated would be effective in inhibiting the muscular atrophy resulting from peripheral nerve injury or anterior poliomyelitis.

CONCLUSION

The muscular atrophy following nerve section in monkeys (Macacus rhesus) can be significantly inhibited by treatment with atropine. This confirms similar results previously obtained on rats, and favors the possibility of a therapeutic application of the drug in humans.

We wish to thank Dr. Philip Lewin for his interest in and his encouragement of this work.

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INFLUENCE OF GELATIN INGESTION ON THE CONCENTRATION IN THE RAT GASTROCNEMIUS OF PHOSPHOCREATINE AND RELATED COMPOUNDS

S. M. HORVATH

From The Harvard Fatigue Laboratory, Harvard University, Boston, Massachusetts

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In a study of the ageing process close attention was paid to the degree of variability, independent of age, that may be expected in the functions studied. It is only after this issue is elucidated that one can certainly ascribe changes observed to the ageing process. For example, what deviations from average values are to be expected when considerable variations are introduced into the dietary regime?

Fortunately for our purpose, a controversy has been going on as to the effects of amino acetic acid or of gelatin, a protein rich in this amino acid, on the concentration of muscle creatine (1). This offered the dual opportunity of testing on the one hand the stability of the chemical system of muscle tissue when a large amount of a particular amino acid is ingested and on the other hand of testing the assumption that amino acetic acid has some unique rôle in creatinogenesis.

METHODS. Adult male rats weighing approximately 200 grams were matched as to size and divided into two groups. Both were fed a stock diet of Purina dog chow, supplemented with green vegetables and powdered skim milk.

To study the excretion of creatine and creatinine, the rats were placed in metabolism cages, two animals in each, for a period of one week before any collections were made. Following this preliminary week, three consecutive 24-hour urine samples were obtained as controls. To the individuals of one group of rats, ½ gram of gelatin in 3 ml. of water was given daily by stomach tube, while to the control group a caloric equivalent of glucose was given in like manner. These supplements were added to the diet for eight days and 24-hour urine samples were collected each day. The urine, free from food and feces, was collected under oil with thymol as a preservative. At the end of each 24-hour period, the cage was washed down with distilled water and the washings added to the urine sample. Aliquots were analyzed for creatine plus creatinine by autoclaving the sample with hydrochloric acid, followed, after neutralization, by the addition of alkaline picrate. Preformed creatine was determined by adding alkaline picrate to fresh urine samples. All color comparisons were made in the Evelyn photoelectric colorimeter.

On the eighth day the animals, having been deprived of food for 12 hours, were anesthetized with nembutal. A gastrocnemius muscle was dissected free and frozen in situ in carbon dioxide snow. The method of sampling the tissue and the procedures employed in the analysis of the tissues have been described (4). Blood was obtained by cardiac puncture.

RESULTS AND DISCUSSION. Many workers have thought that the occurrence of an increased urinary excretion of either creatine or creatinine or both following the administration of various amino acids, other foodstuffs, or hormones, is sufficient evidence of an increased formation of muscle creatine. Beard et al. (2) have reported a marked increase in creatine and creatinine excretion following the injection of a single dose of glycine. As can be seen from table 1, urinary excretion of these compounds by rats was but slightly altered by a high gelatin diet. Averages of all values for the urines collected revealed an increase of only 0.2 mgm. of creatine per day accompanied by a concomitant decreased excretion of creatinine. Variations of this magnitude may have arisen as the result of the inadequate method of collection. Creatinine is readily converted into creatine in some urine samples on standing, even for short periods of time, at ordinary room temperature (3).

TABLE 1

The urinary excretion of creatine, creatinine and nitrogen per rat before and during gelatin feeding

	RATS 3 AND 4		RATS 5 AND 6		RATS 7 AND 8		RATS 9 AND 10		RATS 11 AND 12		RATS 1 AND 2 NOT GIVEN GELATIN
	Before	During	Before	During	Before	During	Before	During	Before	During	
Creatine* mgm. per 24 hours	2.3	4.1	3.7	3.8	4.7	4.1	4.0	4.1	4.4	4.3	3.4
Creatinine mgm. per 24 hours	4.2	4.8	4.5	4.7	8.4	7.4	5.2	4.3	5.7	5.0	3.8
Nitrogen gram per 24 hours	0.17	0.18	0.13	0.17	0.23	0.26	0.25	0.24	0.25	0.25	0.18

* Expressed in terms of creatinine.

Values for the various determinations made on the resting gastrocnemius muscles of 22 rats, fasted for 12 hours, are shown in table 2. Ten of the animals had received ½ gram of gelatin daily for eight days. Of the 22 rats, 6 were from a single litter. Two of these litter mates were in the control group and the remainder in the group receiving gelatin.

Since many of the compounds studied are markedly influenced by even slight activity of the animal, it should be pointed out that these animals were in the resting condition. The concentration of lactate in blood and muscle is regarded as evidence that the animals were in a resting state at the time of sampling. Newman (7) reported respective values of 13 and 22 mgm. per cent lactate in blood and muscle. Average values of blood and muscle lactate in the present study were 10 and 16 mgm. per cent in the gelatin group and 13 and 19 in the control group.

There was no significant difference between the control and gelatin groups in any of the substances studied. In general, agreement was excellent not only

between litter mates, but also among all other rats. A critical evaluation of the influence of the ageing process on the distribution of the phosphate and related compounds has been presented (4).

The two compounds in muscle which are supposed to be affected by the addition of gelatin to the diet are the total creatine and that part of it bound in the form of phosphocreatine. According to Ray et al. (8) gelatin added to the diet had a marked influence on the physical capacity of man. The allaying of muscular fatigue and the increased work output was attributed to the creatinogenic effect of the amino acetic acid portion of the gelatin with the implication that there was an actual increase in phosphocreatine. If phosphocreatine in the muscle increases, it might serve as an additional source of muscular energy.

TABLE 2

Mean composition of adult rat tissue in relation to gelatin feeding*

Total solids and total nitrogen are in terms of grams per cent in wet tissue; all other concentrations are in milligrams per cent.

	RATS ON STOCK DIET	RATS ON STOCK DIET PLUS 3 GRAMS GELATIN DAILY FOR 8 DAYS		RATS ON STOCK DIET	RATS ON STOCK DIET PLUS 3 GRAMS GELATIN DAILY FOR 8 DAYS
Number of animals	12	10	Adenosine triphosphate	38	39
Total solids	25.0	25.5	Hexose phosphates	19	22
Tissue nitrogen	2.1	2.4	Barium insoluble phosphate	77	77
Total creatine	406	396	Glycogen	590	525
Total phosphate	228	232	Muscle lactate	19	16
Acid soluble phosphates	154	155	Blood lactate	17	10
Inorganic phosphate	19	20	Blood sugar	129	132
Phosphocreatine	58	56			

* Eight to nine months old.

Possibly it would facilitate a more rapid resynthesis of adenosine triphosphate, which is believed to furnish the immediate energy for muscular contraction.

No changes in either phosphocreatine or total creatine were found as the result of gelatin ingestion by the rats in this study. The phosphocreatine concentrations were 56 and 58 mgm. per cent respectively for the gelatin and non-gelatin groups, while the total creatine concentrations were 396 and 406 mgm. per cent. This finding that gelatin has no positive effects is in accord with the observations of Horvath et al. (5) and of Robinson and Harmon (9) that gelatin added to the diet of men did not alter their strength or endurance. Knowlton (6) found that gelatin did not affect the strength of the leg muscles of rats.

SUMMARY

The feeding of gelatin, a rich source of amino acetic acid, to adult rats as a supplement to an adequate diet did not influence the concentration in the gastrocnemius muscle of the chemical compounds known to be associated with

muscular contraction. Gelatin exerted no creatinogenic effect. No changes were found in either phosphocreatine or total creatine. There was no evidence on the basis of increased excretion of creatine or creatinine of an increased destruction of muscle creatine.

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THE RELATION OF FOOD CONSUMPTION, HYPOPHYSIS AND ADRENAL CORTEX TO SERUM ALBUMIN METABOLISM IN THE RAT[1]

LOUIS LEVIN

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York

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In a previous communication (1) it was suggested that the decrease in serum-albumin concentration following hypophysectomy in rats is due mainly to decreased adreno-cortical function. The self-imposed inanition which follows removal of the hypophysis also has an influence. However, that this is of relatively minor importance in the short experimental periods employed was demonstrated (1) by the finding that in intact female rats, subjected to an inanition somewhat more severe than that suffered by the operated animals, the serum-protein changes were of much smaller magnitude than those found after hypophysectomy. Moreover, hypophysectomized rats treated with adrenocortical substances were found to maintain their serum albumin at a fairly normal level even though the loss of body weight was as great as in untreated, operated animals.

These findings indicate that under the influence of adrenocortical hormone the rat is able to maintain its serum-albumin level, if necessary, at the expense of other body proteins. However, if adrenocortical function is decreased or entirely absent, the maintenance of the normal serum-albumin level by utilization of endogenous protein does not appear possible. It was, therefore, of interest to know whether animals deprived of most or all of their adrenocortical function are able to maintain the serum-albumin level if sufficient food is supplied by mouth, or in other words, whether adrenocortical intervention is necessary for the formation of serum albumin from exogenous protein. To this end we have determined the serum-protein levels of normal and of hypophysectomized rats supplied with adequate amounts of protein by stomach tube. The results were compared with those obtained from similar animals eating *ad libitum*. In addition, to check our previous findings in female rats, we determined the serum-protein levels in intact male rats subjected to severe inanition.

The results of these investigations, reported in this communication, show that the serum albumin of the hypophysectomized rat remains at very nearly the normal concentration if the food intake is maintained at a sufficiently high level.

METHODS. Adult male rats were selected and divided so that the different experimental groups matched approximately in age and weight. Two of the groups were hypophysectomized while the other two remained unoperated. One of the hypophysectomized and one of the intact groups received no treatment of

[1] Aided by a grant from the Rockefeller Foundation administered by Dr. P. E. Smith.

any sort, being allowed to eat the regular stock diet[2] *ad libitum*. The other two groups, one of operated and one of intact animals, were fed by stomach tube, usually beginning about 36 hours after the operation. The tube feeding was done twice daily by a method described elsewhere (2), using increasing amounts of the food mixture [3] during the first few days so as to minimize danger of food shock. These animals therefore lost weight for several days until the amount of food given was sufficient to supply the caloric requirements. The same amount of food was administered to the normal and operated animals regardless of weight or other factors.

In addition to the above, another group of intact male rats was subjected to a reduced food intake (using the regular stock diet) so as to simulate the weight loss suffered by the hypophysectomized animals eating *ad libitum*.

All the experiments were conducted over a period of 3 weeks. At the end of this period the animals were bled from the heart and the sera analyzed by methods previously described (1). The completeness of hypophysectomy was verified at autopsy by careful examination of the pituitary region with the aid of a high power dissecting microscope. In addition, the testes, seminal vesicles, adrenals

[2] The stock diet used for our rat colony is composed of the following ingredients which are thoroughly mixed and fed *ad libitum* as the dry mixture. A supplement of fresh lettuce is fed once weekly and water is available to the animals at all times.

Ground yellow corn	15 parts (by weight)
Ground whole wheat	15 parts
Ground hull-less oats	15 parts
Ground whole barley	15 parts
Soy bean meal	15 parts
Meat scrap	10 parts
Whole milk powder	5 parts
Skim milk powder	5 parts
Ground alfalfa meal	2 parts
NaCl	2 parts
$CaCO_3$	0.5 parts

[3] The liquid diet used for tube feeding in these experiments is prepared as follows:

Whole milk powder	300 gm.
Dried egg albumin	40 gm.
Dried irradiated yeast	50 gm.
Cellu flour	60 gm.
Salt mixture (Osborne Mendel)	10 gm.
Glucose	115 gm.
Mazola oil	45 cc.
Wheat germ oil	5 cc.
Cod liver oil	5 cc.
Vitamin B syrup[4] (Abbott)	15 cc.
Water	500 cc.

The glucose is dissolved in the water and the solution is added to the mixed dry ingredients. The mixture is stirred with an egg beater, adding the oils and the vitamin B preparation. Stirring is continued until the mixture is homogeneous. This mixture was administered in daily doses of 24 cc., divided into two feedings. This amount (24 cc.) is calculated to contain 2.9 grams protein, 5.5 grams carbohydrate and 3.2 grams fat as available foodstuffs. The yeast was omitted from the calculations.

[4] The vitamin B syrup was kindly furnished by Dr. D. W. MacCorquodale of The Abbott Laboratories.

and thyroids were dissected and weighed. The atrophy of these organs during the three-week post-hypophysectomy period is sufficiently great to allow dependable judgement as to the completeness of pituitary removal.

RESULTS AND DISCUSSION. The changes in body weight of the different experimental groups are charted in figure 1. It is evident that the tube-fed hypophysectomized rats, after the immediate 2 or 3 day postoperative period, gain weight at a rate identical with that of the intact animals given the same amount of food. The greater loss of weight suffered by the operated animals during the first 2 days is probably due to the effects of anesthesia, surgical procedures, etc. It is of considerable interest to note that the rate of weight increase of the

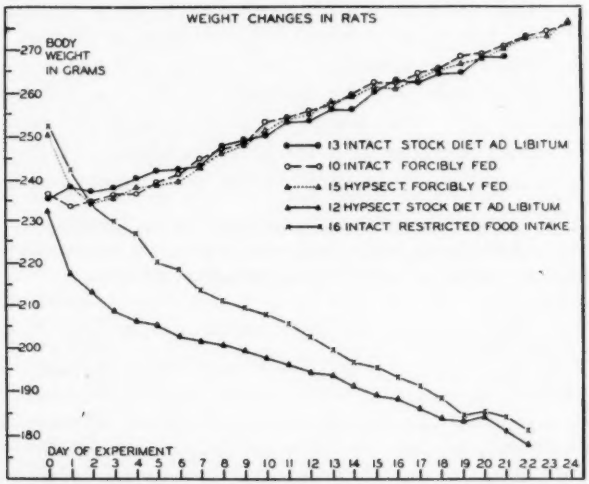

Fig. 1. Relation of food consumption, hypophysis and adrenal cortex to serum albumin metabolism in the rat.

force-fed animals, hypophysectomized as well as intact, is identical with that of intact animals eating the stock diet *ad libitum*. It would be of interest to know whether the composition of this weight increment is the same for the 3 types of animal. That this is so is indicated by the findings of Samuels et al. (3), but would not be expected in the light of the work of Lee and Ayres (4).

The analytical data obtained from the sera of the above described animals are summarized in table 1. The changes found in the hypophysectomized animals eating *ad libitum* (1, C) agree well with those previously reported (1) for such animals, being a marked decrease in serum albumin and an equally marked increase in serum-globulin concentration. Likewise, severe inanition in intact male rats produces changes (1, B) similar to those previously found in starved female rats. These changes are qualitatively similar but quantitatively of much less magnitude than those seen after hypophysectomy. This indicates that the hypophysectomized rat, deriving a considerable proportion of its caloric requirements from endogenous sources (as shown by the marked loss of body

weight), is not able to replenish or replace its serum-albumin stores. On the other hand, the intact rat, if forced to subsist to an equal extent on its endogenous resources as a result of curtailed food intake, is able to maintain its serum-albumin concentration at a more nearly normal level.

This difference in ability to maintain the serum albumin level disappears if an amount of food, approximately isocaloric with that which is voluntarily consumed by the intact rat, is administered to the hypophysectomized as well

TABLE 1

Effect of various treatments on serum protein levels of the rat

TREATMENT	NO. OF RATS	AGE AT BLEEDING	DURING EXPT.		CELL VOLUME	NON-PROTEIN NITROGEN	TOTAL PROTEIN	ALBUMIN	GLOBULIN	ALBUMIN / GLOBULIN
			Time	Wt. chg.						
		days	*days*	*per cent*	*per cent*	*mgm. per cent*	*per cent*	*per cent*	*per cent*	*ratio*†
A. Normal—stock diet ad lib.	18	112–138	15–22	+12.2±1.2	44.6±0.5	33.8±1.0	6.04±0.07	4.01±0.03	2.04±0.06	1.99±0.06
B. Intact—restricted food intake	16	124–134	20–22	−27.0±0.9	45.5±0.7	35.6±1.2	5.63±0.08	3.57±0.07	2.06±0.08	1.78±0.08
Per cent change from normal					+2.2	+5.3	−6.8	−11.0	+1.0	−10.5
P* (vs. normal)					0.30	0.25	<0.01	<0.01	0.80	0.05
C. Hypsect.—stock diet ad lib.	12	112–129	21–22	−21.1±2.0	40.9±1.4	46.5±1.5	5.57±0.15	3.07±0.07	2.50±0.10	1.24±0.05
Per cent change from normal					−8.3	+37.6	−7.8	−23.4	+22.6	−37.7
P* (vs. normal)					<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
D. Intact—force-fed	10	128–138	19–24	+15.4±1.7	42.7±3.5	30.0±1.7	5.85±0.15	3.70±0.08	2.15±0.11	1.76±0.09
E. Hypsect.—force-fed	15	128–138	19–24	+7.9±1.0	36.5±1.0	44.6±0.7	6.20±0.10	3.52±0.08	2.68±0.07	1.32±0.04
Per cent change from normal force fed					−14.5	+48.7	+6.0	−4.9	+24.7	−25.0
P* (vs. intact force fed)					<0.01	<0.01	0.07	0.15	<0.01	<0.01
Per cent change from hypsect. ad lib.					−10.8	−4.1	+11.3	+14.7	+7.2	+6.5
P* (vs. hypsect. ad lib.)					0.02	0.25	<0.01	<0.01	0.15	0.22

The ± values are for the mean deviation of the mean, calculated as $\sigma_m = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

*P expresses the probability that the difference between two means is due to random sampling (11). A P value of 0.05 or less is frequently taken to indicate statistical significance.

† The values given for the A/G ratio are the means of the individual A/G ratios rather than the ratio of the mean albumin to mean globulin.

as to the normal animal. As shown by the data of table 1, D, intact rats receiving their entire food supply by stomach tube yield results comparable to those obtained from normal animals eating *ad libitum* (table 1, A). Hypophysectomizde rats, given this same amount of food by stomach tube, maintain the serum-albumin concentration at approximately the normal level (table 1, E). The ratio of albumin to globulin, however, is low because the forced feeding does not prevent the sharp increase in serum globulin which is found after hypophysectomy or thyroidectomy in the rat (1) and in the dog (5). It may be noted that the hematocrit level of the forcibly fed hypophysectomized animals is considerably lower than that of the intact rats. Although the reason for this is not known to us, it is suspected that it is due to decreased hemopoiesis (6). If, however, the

low hematocrit is due to a hemodilution in the sense of increased plasma volume, then the serum-albumin maintenance is even better than shown by the figures.

These results are interpreted to mean that little or no adrenocortical hormone is required for the maintenance of the serum-albumin level if sufficient food is supplied to the animal. However, if the animal is forced to subsist wholly or in part on its endogenous resources, adrenocortical or hypophyseal biocatalysis is required for the maintenance of the serum-albumin level. Previous experiments (1, 7) provide evidence that the necessary hormonal action is adrenocortical rather than hypophyseal.

The situation with regard to serum-albumin maintenance, therefore, appears to be analogous to but more extreme than that concerning maintenance of carbohydrate stores. It has been adequately shown (8, 9) that adrenalectomized or hypophysectomized animals are able, under ordinary circumstances, to maintain fairly normal carbohydrate stores despite the sharply decreased food consumption. Only under conditions of stress, when carbohydrate must be rapidly formed from endogenous protein, does the gluconeogenic mechanism break down.

Similarly, serum albumin apparently can be manufactured satisfactorily from exogenous protein with the reservation that the supply of this raw material must be considerably greater than that which the operated animal voluntarily consumes. When the supply of exogenous protein is decreased, as in the hypophysectomized or adrenalectomized animal eating *ad libitum*, the replacement of serum ablumin is greatly impaired. However, if the animal has an adequate supply of adrenocortical hormone it apparently is able to maintain its serum-albumin level at the expense of other body proteins, at least for periods as long as those studied in the present experiments. That the mechanism may ultimately break down, at least in some species, is known (10).

The author gratefully acknowledges the important technical assistance of Miss Shirley Mischler and Miss Betsy Conant.

SUMMARY AND CONCLUSIONS

1. Following hypophysectomy, rats voluntarily decrease their food consumption and show a marked loss of body weight. Concomitantly, there occurs a marked decrease in the serum-albumin concentration.

2. Starvation of intact rats to simulate the body-weight loss of the hypophysectomized animals also produces a decrease in serum albumin but this is of much less magnitude than that occurring in the operated animals.

3. If adequate quantities of food are forced into hypophysectomized adult rats by stomach tube, they can be caused to gain weight at a rate comparable to that resulting from *ad libitum* feeding of normal animals. In such force-fed hypophysectomized rats, the serum-albumin concentration is maintained at the normal level.

4. These and previous findings indicate that with adequate adrenocortical function the rat is able to maintain its serum-albumin concentration, at least for periods as long as three weeks, even when forced to subsist partially on its

own tissues. In the event that adrenocortical function is decreased or absent, the rat is unable to maintain its serum albumin at the normal level unless it is supplied with quantities of food considerably larger than hypophysectomized or adrenalectomized animals voluntarily consume.

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THE ANTAGONISTIC EFFECT OF LIPOCAIC AND THE ANTERIOR PITUITARY ON FAT METABOLISM[1]

ORMAND C. JULIAN,[2] DWIGHT E. CLARK, JOHN VAN PROHASKA, C. VERMEULEN AND LESTER R. DRAGSTEDT

From the Department of Surgery of the University of Chicago

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There is now general agreement that the activity of raw pancreas in preventing the deposition of fat in the livers of depancreatized dogs is not due to lecithin, choline, betaine, the enzymes of the pancreatic juice or to the general lipotropic action of protein. This activity is due to a specific substance, lipocaic, and the evidence indicating that this is a hormone distinct from insulin has been enumerated by Dragstedt (1). The suggestion by Best (2) that lipocaic is not a hormone but rather a specific dietary factor is probably a matter of definition.

The mode of action of lipocaic, except that in a general way it is concerned with the transport and utilization of fat, has remained relatively obscure. The characteristic changes in lipocaic deficiency: loss of body fat, accumulation of liver fat in large amounts, and hypolipemia are observed in depancreatized dogs in whom the other specific defects are corrected by the administration of pancreatic juice and insulin. These findings suggest a transport of depot fat to the liver and accumulation there due either to the increased rate of mobilization of fat or to an inability of the liver to further metabolize it. The administration of lipocaic corrects this metabolic abnormality in the depancreatized dog.

Abnormalities in fat metabolism similar to those seen in the insulin-treated depancreatized dog can be produced in smaller animals by the administration of certain anterior pituitary extracts. The first extract of this kind was prepared in 1930 by Burn and Ling (3). They termed the material ketogenic hormone because when they administered it to rats an increase in the urinary excretion of ketones was observed. The action of this substance was further studied by Anselmino and Hoffman (4) who found an accompanying increase in the blood ketones and in 1934 by Steppuhn (5) who showed that there was an increase in the fat content of the liver of rats receiving injections of the ketogenic hormone. Best and Campbell (6) studied the production of this type of fatty liver. In 1936 they confirmed the previous observations and noted that accompanying the increase in liver fat there was a decrease in the amount of body depot fat in fasted animals given the extract and that this decrease was greater than the decrease seen in the control fasted animals. The fat lost from the depots of the body was found to account for about one-half of the lipid recovered on analysis of the liver.

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[2] Submitted by Dr. Julian to the University of Chicago in partial fulfilment of the requirement for the degree of Ph.D. in surgery.

Best (7) had previously confirmed earlier work showing that large doses of choline would prevent the fatty liver of depancreatized dogs. Consequently, he tested the effect of choline on the fatty liver produced by anterior pituitary extracts and found no effect.

The purpose of the present study was to observe the effect of administration of lipocaic on the fatty liver produced by the injection of pituitary extracts. Further experimental work suggested by the results of this study is also reported.

This type of experiment had previously been made by McKay and Barnes in 1938 (8). These workers tested the effect of both choline and lipocaic on the ketogenic hormone fatty liver. The conclusion from their data was that neither substance had any effect. However, the pancreas extract was given in amounts which seemed to us to be too small to expect definite activity. They prepared lipocaic according to the method given in 1936 by Dragstedt, Prohaska and Harms (9), and, using rats, administered an amount of this extract equivalent to 8.7 grams fresh pancreas per square decimeter of body surface daily. This is approximately 30 grams fresh pancreas per day for a rat weighing 175 grams.

In the present study the lipocaic was purified for small animal work by the glacial acetic acid-ether method reported by Clark, Vermeulen, Donovan, and Dragstedt in 1939 (10). This method yields an average of 100 mgm. of extract from 200 grams of raw pancreas. In the work to be presented, daily doses of this extract ranging from 25 to 250 mgm., equivalent to 50 to 500 grams of raw pancreas, were given each animal. The preparation of the ketogenic hormone followed the method of Best and Campbell.

Three groups of young female guinea pigs weighing 300 to 375 grams were used. The initial weights were determined. All animals received only water by mouth during the 72-hour experimental period. Each animal in the three groups received an intraperitoneal injection after 24 hours and again after 48 hours of fasting. They were killed 24 hours after the second injection.

The animals in the control group received injections of 3 cc. of saline. The animals of the second group (group B) were given ketogenic hormone in doses varying from 2 to 10 mgm. per 100 grams of body weight. The animals of group C received similar amounts of ketogenic hormone plus total daily doses of 25 to 250 mgm. of lipocaic injected intraperitoneally at separate sites. At the completion of the 72-hour period the loss in the body weight, the final liver weight, and the percentage of lipid in the liver were determined. The total liver lipids and the milligrams of liver lipids per 100 grams final body weight were calculated. The results of these determinations are presented in table 1.

The average loss of body weight was greatest in both groups of animals receiving ketogenic hormone. This weight loss was not affected by the administration of lipocaic. The livers of the animals receiving ketogenic hormone were heavier than those that received additional lipocaic and than those of the control group.

The average percentage of liver lipids was found to be 11.1 in the control animals, 18 in the ketogenic hormone group, and 8.1 in the animals receiving lipocaic with the ketogenic hormone. The total liver lipids followed the same

pattern being 1138 mgm., 2677 mgm. and 1041 mgm. respectively in the three groups. To relate these figures to the sizes of the animals, the milligrams of liver lipid per 100 grams of final body weight are given. These calculated figures show very strikingly the protective action of lipocaic against the effect of ketogenic hormone.

This demonstration of an antagonistic relationship between lipocaic and the pituitary extract, ketogenic hormone, is important from the standpoint of the mechanism of lipocaic deficiency in the depancreatized dog. Does the insulin treated depancreatized dog develop a fatty liver as a result of the action of the anterior hypophysis, unopposed by lipocaic?

TABLE 1

Antagonistic effect of lipocaic on the fatty infiltration of the liver produced in guinea pigs by fasting and the injection of ketogenic hormone

NO. OF ANIMALS	AVERAGE INITIAL WT.	AVERAGE FINAL WT.	AVERAGE LOSS IN WT.	AVERAGE LIVER WT.	PER CENT LIVER LIPID			MGM. TOTAL LIPID IN LIVER			MGM. LIVER LIPID PER 100 GM. FINAL BODY WT.		
					High	Low	Aver.	High	Low	Aver.	High	Low	Aver.
Control group—3 cc. saline intraperitoneally													
	gm.	*gm.*	*gm.*	*gm.*									
6	349	298	51	9.9	16.5	5.2	11.1	1575	499	1138	496	172	375
Group B—1.5 to 10 mgm. ketogenic hormone per 100 gm. of animal intraperitoneally													
19	341	274	62	14.8	27.9	11.2	18	4525	1710	2677	1932	540	1011
Group C—1.5 to 10 mgm. ketogenic hormone per 100 gm. plus total dose of 25 to 250 mgm. of lipocaic preparation													
21	349	293	60	12.7	12.0	4.3	8.1	1480	425	1041	610	175	364

In order to examine further the rôle of the hypophysis in the fatty liver production in pancreatectomized dogs, a series of animals was first hypophysectomized and then depancreatized.

The results of this study are given in table 2. The dogs, nos. 1 to 6, survived the second operation from eleven to forty-two days. At autopsy the livers showed uniformly a marked increase in fat content, ranging from 22 to 42 per cent.

Dogs 7 to 10 were subjected to liver biopsy seven to forty-nine days after pancreatectomy. After biopsy the dogs were given lipocaic by mouth in doses known to cure fatty liver in pancreatectomized dogs. Following lipocaic administration for eight to one hundred and five days the dogs were sacrificed, usually at an attempted second biopsy, and a uniform decrease in liver fat observed. The liver of one of these dogs (no. 10) was biopsied after thirty-five days following pancreatectomy at which time the liver assay showed 43 per cent lipid. A second biopsy forty-one days later, lipocaic being given in the interval, showed 12 per cent fat in the liver. The dog died thirty-one days

after this second biopsy, and at this time a normal fat content of the liver was found.

The depancreatized, hypophysectomized dog develops a severe fatty liver in as short or shorter time than the dog with pancreatectomy alone. This fact has been infrequently observed in this preparation because most investigators using the Houssay animal have fed raw pancreas, containing lipocaic, as a food supplement.

This rapid appearance of the fatty liver in the Houssay animal indicates that the fat metabolism factor of ketogenic hormone is not the only factor in the

TABLE 2

Development of fatty infiltration of the liver in the hypophysectomized-depancreatized dog (Houssay) and response to lipocaic therapy

DOG	OPERATION DATES		DATE OF DEATH	LIVER FAT PER CENT WET WT.
	Hypophysectomy	Pancreatectomy		
				per cent
1	6/4	9/2	10/2	27
2	10/5	10/26	11/19	35
3	11/20	12/16	12/29	22
4	12/15	12/30	2/14	38
5	12/18	2/5	2/20	32
6	10/15	11/4	11/15	42

DOG	OPERATION DATES		LIVER BIOPSY		TREATMENT	LIVER POST-MORTEM	
	Hypophysectomy	Pancreatectomy	Date	Fat		Date	Fat
				per cent			*per cent*
7	11/26	12/3	1/3	32	Lipocaic 16 days	1/10	11
8	12/30	3/31	4/7	12	Lipocaic 8 days	4/15	9.9
						Perforated ulcer	
9	12/24	2/24	4/13	18	Lipocaic 3½ mo.	6/24	11
10	11/19	12/3	1/8	43	Lipocaic 2½ mo.	3/20	Normal liver
			2/19	12			

appearance of fatty liver in the depancreatized dog, though it may play a part in the lipid metabolism of the intact animal.

Discussion. The antagonistic action of lipocaic and certain extracts of the anterior pituitary suggests that under normal conditions the transport of fat between the body depots and the liver is regulated by opposing hormone influences. It seems probable that the anterior pituitary liberates a substance into the circulation which causes a migration of body fat to the liver. Excessive breakdown of this fat results in hyperketonemia and ketonuria. Lipocaic, on the other hand opposes at least a part of this effect and causes the migration of fat to the body depots. Evidence with respect to the effect of lipocaic on the ketonemia and ketonuria resulting from injection of the ketogenic hormone is

not available. While the accumulation of fat in the liver of the depancreatized dog may be in part due to the unopposed action of the anterior pituitary in the absence of lipocaic, the fact that this accumulation occurs with equal rapidity in the "Houssay" animal indicates that this is not the sole mechanism.

CONCLUSIONS

1. The parenteral administration of lipocaic prevents the accumulation of fat in the liver produced by the injection of ketogenic hormone in fasting guinea pigs.

2. The fatty infiltration in the liver due to fasting is also decreased by the administration of lipocaic.

3. The Houssay animal develops the fatty liver of lipocaic deficiency just as rapidly as the depancreatized dog and is equally responsive to lipocaic therapy.

4. Some implications of the antagonistic action of lipocaic and the anterior pituitary on fat transport are mentioned.

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STUDIES ON INTRAOSSEOUS INJECTIONS OF EPINEPHRINE

DAVID I. MACHT

From the Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Maryland

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In the last two or three years blood has been successfully transfused in both animals and human beings by injection into the medullar cavity of long bones. Particularly meritorious work along this line has been published by two sets of American clinical investigators. Morrison and Samwick (1) reported successful intramedullar transfusion not only of blood but of human bone marrow cells, while Tocantins and O'Neill (2) injected whole blood, blood plasma, glucose solution and congo red through the bone marrow. Such hematological experimentation prompted an inquiry by the present author into the feasibility and effects of intramedullar injection of different pharmacological agents on various animals. Fifty experiments were made on guinea pigs, forty on rabbits, thirty on cats, five on dogs and twenty on rats. In many instances such experiments yielded striking results, which have been described in a preliminary paper (3) on the absorption of drugs through the bone marrow. In the present communication the writer purposes to discuss in detail one drug, epinephrine, which has yielded experimental data of unusual interest to both physiologist and pharmacologist.

METHOD. Intramedullar injections are readily made after some practice by introducing stout needles or trocars into the long bones of rabbits, cats, guinea pigs, dogs and rats. The most convenient bone for this purpose is the tibia which in rabbits and smaller animals can be pierced without difficulty by needles varying in proportion to the rodents' size. In larger animals, i.e., cats and dogs, the author employed special trocars or metal cannulae, which were inserted tightly into apertures bored through the bones with a fine drill or, more conveniently, with a sharp awl. The results obtained with epinephrine and also with ephedrine, a closely related drug, may be conveniently described under two headings, namely, experiments with aqueous solutions and experiments with oil suspensions.

Experiments with aqueous solutions. Injections of aqueous solutions of epinephrine into medullar cavities of the long bones of various animals produced unexpected results. The pharmacodynamic effects of such injections are hardly distinguishable from those following intravenous administration of similar solutions of the suprarenal medulla hormone. There is an immediate sharp rise in blood pressure of short duration followed by a rapid fall to below normal level and then a gradual return to normal level. As the subjoined illustration indicates, epinephrine in aqueous solutions is absorbed almost as rapidly after intraosseous injection as after administration by vein (fig. 1).

Subcutaneous and intramuscular injections of aqueous solutions of epinephrine

produced little or no rise in blood pressure of ordinary laboratory animals although clinical practitioners by such means have effected bronchodilatation in cases of bronchial asthma. More recently, certain clinicians using suspensions of epinephrine in oil in the treatment of bronchial spasm have claimed that their effect lasts longer than that produced by aqueous solutions of the hormone (4). While that is true with regard to the effect of epinephrine on the bronchi, blood pressure experiments on cats, rabbits and dogs have revealed little, if any, pressor effect on the blood pressure curve after hypodermic or intramuscular injection of either aqueous or oil solutions of epinephrine.

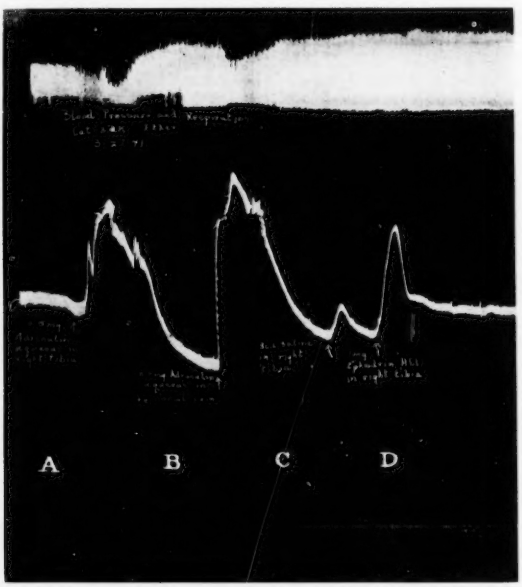

Fig. 1. Blood pressure and respiration of cat weighing 3.2 kilos under ether.
A. Injection of 0.5 mgm. of aqueous adrenalin in right tibia.
B. Injection of 0.5 mgm. of aqueous adrenalin in femoral vein.
C. Injection of 3 cc. of physiological NaCl solution in right tibia.
D. Injection of 1 mgm. of aqueous solution of ephedrine HCl in right tibia.

Experiments with oil suspensions. Very different results from those obtained with aqueous solutions were derived from experiments in which suspensions of epinephrine in oil were introduced into the medullar cavities of the long bones. Slow injections of a small quantity of adrenalin suspension, varying from 0.1 to 1.0 cc., depending on the size of the animals, were followed promptly by a sharp rise of the blood pressure to a moderate height, due undoubtedly to direct absorption of some of the oil suspension into the neighboring vessels. This rise in blood pressure is only half and sometimes less than half that noted after intraosseous injections of aqueous solutions, indicating that but a small amount of the suspension had been absorbed into the neighboring vessels.

On the other hand, the rise in blood pressure was not succeeded by the rapid return to normal level noted after both intravenous and intraosseous administration of aqueous epinephrine solutions. On the contrary, the pressor effect of epinephrine continued to be exerted for some time after its injection and in some cases lasted for as long as an hour (fig. 2). This remarkable sustained pressor effect of injections of epinephrine in oil into the medullar cavities is of special interest in connection with two problems immediately suggesting themselves to the physiologist. What is the explanation of this peculiar sustained effect and what is the incidence of oil embolism in such experiments?

In an endeavor to solve the second problem, the writer carried out a large number of control experiments with pharmacologically inert fixed oils, i.e., olive oil, peanut oil, peach kernel oil, sesame oil, cottonseed oil and mineral oil. Intramedullar injection of small quantities of such oils in the long bones of all the animals studied was usually harmless. Dangerous oil embolism occurred but rarely and deaths from such experiments with the fixed oils were exceptional

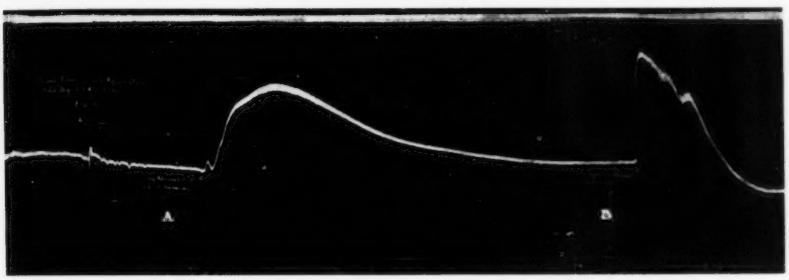

Fig. 2. Blood pressure and respiration of male dog, weighing 5.5 kilos, under ether.
A. Injection of 1 mgm. of adrenalin in oil in right humerus.
B. Injection of 1 mgm. of aqueous adrenalin in left humerus.

although their intravenous injection was usually fraught with great danger. It appears that the injection of such heavy or fixed oils into the medullar cavities is followed by but very slow absorption of the oil into the circulation, possibly in a more or less emulsified state. The prolonged action of epinephrine in maintaining a high blood pressure level in such experimental animals may be explained in the same way. These oil solutions of epinephrine apparently remain for considerable periods in the medullar cavities, which act as reservoirs for a drug that is slowly liberated and dispensed by the oil and passed into the circulating blood, where its pressor effect is maintained for a long time.

The comparatively innocuous effects of the fixed oils employed in the control experiments must not be confused with the effects of the so-called essential or volatile oils, which differ widely from the fixed oils in chemical composition, physical properties and physiological effects. All these essential oils, the writer has found, are rapidly absorbed through the skin and mucous membranes of small and large animals, producing toxic effects (5, 6). A similar action is rapidly exerted by the essential oils after their injection into the bone marrow.

The pharmacological or toxicological picture obtained with all of them is characteristic. There is a primary stage of excitement and agitation, followed by a depression and paralysis of the central nervous system and, finally, by coma, convulsions and death if sufficiently toxic doses of the oils be employed.

COMMENT. As compared with that following intravenous injection of aqueous solutions of epinephrine, the rapidity of absorption of such solution from the bone marrow is of special interest. Experiments reveal that aqueous solutions of adrenalin thus injected are absorbed almost as rapidly as when administered by vein. This phenomenon, observed by the writer after administration of aqueous solutions of all kinds of drugs, is confirmed by the enthusiastic reports of hematologists with regard to effective transfusion of blood plasma and whole blood in human beings. The curious findings obtained with intraosseous injections of epinephrine solutions *in oil* are of primary interest to the experimental biologist. Even though the writer's studies with intraosseous injection of fixed oils revealed that but a few cases of fatal embolism or gross pathological lesions are produced by such a procedure, it would be hazardous to draw hasty conclusions of a therapeutic nature from such experimentation and attempt to inject oil solutions of active principles into the medullar cavities of human beings before a great deal of further investigation had been completed with regard to the anatomy, physiology and pathology of the human osseous, vascular and hematopoietic systems as compared with those of lower animals. The cautious practitioner will always be mindful of the difficulties connected with the translation of observations on animals into clinical potentialities. The findings obtained with epinephrine, of course, are of great interest in connection with a study of certain phases of shock, and it is the writer's hope that they will stimulate further research along this line by both laboratory and clinical investigators.

SUMMARY

1. Intramuscular and hypodermic injections of both aqueous and oil solutions of epinephrine exert little or no effect on blood pressure of experimental animals.

2. Intraosseous injections of aqueous solutions of epinephrine are followed by immediate absorption of the hormone, as indicated by a prompt rise in blood pressure succeeded by a rapid fall to below normal level, effects which are not distinguishable from those following intravenous injection of such solutions.

3. Intramedullar injection of epinephrine suspensions in oil is followed by a prompt rise in blood pressure to a moderate level, long sustained, the maintenance of the pressure for a long time at that height indicating the slow and continuous absorption of this drug from the bone cavity.

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THE INITIATION OF IMPULSES IN CARDIAC MUSCLE

EMIL BOZLER

From the Department of Physiology, the Ohio State University, Columbus

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Although the process of the initiation of impulses in the vertebrate heart has engaged the interest of physiologists for a long time, only few attempts have been made to study this problem directly and with negative results (1, 7). However, Arvanitaki (3), although working on entirely different material, the snail's heart, observed weak potential changes preceding the discharge of impulses. These results agree in their principal aspects with those described below for the vertebrate heart and with previous observations on visceral smooth muscles (6). In all these muscles weak non-conducted potentials initiate the spontaneous impulses. These local potentials, which resemble those of partly decalcified and spontaneously discharging nerve and skeletal muscle fibers (2, 3, 4), must be considered the basis underlying automaticity and will be called prepotentials.

A particularly interesting phenomenon are potential oscillations which, under certain conditions, initiate and follow impulses. Although these local potentials occur only in injured muscle they deserve attention because they explain some peculiar rhythmic phenomena previously described in isolated cardiac muscle. Furthermore, the regularity of the oscillations suggests that they express an important property of the tissue.

METHODS. Strips from different parts of the heart of large turtles, rabbits, cats and dogs were used in most experiments. Monophasic potentials were obtained, as described previously for intestinal muscle (6), by forcing one end of a preparation inside a narrow glass tube and by attaching one of the leads to the part of the muscle thus inactivated. Diphasic artifacts were not always completely excluded by this technique but the potentials usually remained constant for several hours. A Toennies d. c. amplifier was used. Records were made either by a mechanical recorder writing on a smoked drum or by an oscillograph. It should be emphasized that the maximal height of the conducted potentials is not correctly indicated by the records because the impulses are too large (usually 5 to 10 mVolt) as compared with the local potentials and are, therefore, cut off by the amplifier.

RESULTS. A. *Prepotentials.* To determine the processes which precede the discharge of an impulse, potentials were led off monophasically from the pacemaker. The precise origin of the impulses in a spontaneously beating muscle strip was found by placing the leads close together and by determining the point where the direction of the deflection reverses itself if the leads are successively shifted in one direction along the preparation. The correctness of this procedure, previously applied to the ureter, was confirmed by the fact that the location of the pacemaker so determined agreed with the region where the first

visible movements occurred when the preparation was removed from a refrigerator and was gradually warming up.

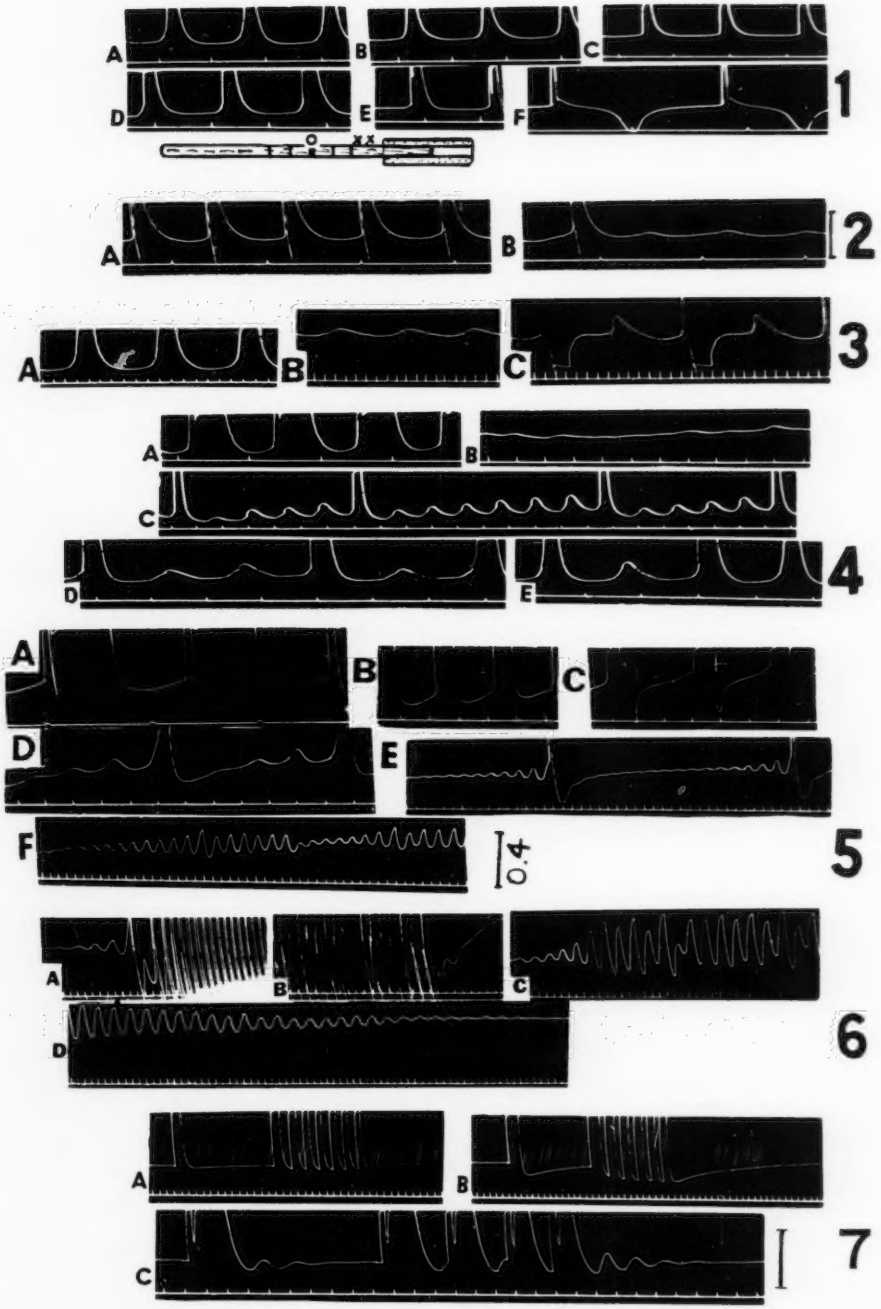
While the muscle shows normal rhythmicity each impulse is preceded by a period of gradually rising negativity. The significance of the local potentials is still more strikingly demonstrated in preparations in which the discharge is induced by very regular potential oscillations. These two phenomena will be described separately.

1. *Local potentials during normal rhythmicity.* If the active lead was placed on the origin of the impulses, potentials as shown in fig. 1, 2, 3A, 4A, 5A were usually obtained. A gradual rise in negativity preceded each impulse. That this phase of the action potential, amounting to 0.2 mVolt or less, is not due to the conduction of the impulse is shown by the fact that the ascent of the curve is sharp if both leads are on intact tissue and at some distance from the pacemaker (fig. 1F).

The phenomenon is, in many cases at least, partly due to a diphasic artifact or the disappearance of a positive after-potential (p. 7). However, it is significant that the rise in negativity at the pacemaker becomes progressively faster just before the discharge, whereas it would be expected to level off if it were entirely due to an after-effect of the previous impulse. The last phase of the potential change may, therefore, be interpreted as a prepotential.

This interpretation is confirmed by the observation that the size of the potential decreases on both sides of the pacemaker as shown in figure 1 and previously demonstrated for the ureter. In the turtle the prepotentials can be observed in the whole sinus region and their magnitude may be nearly the same over a distance of about 5 mm. This result agrees with the fact that the whole sinus possesses a considerable degree of automaticity and that, apparently, the precise origin of the impulses is determined by small quantitative differences between different regions. Sometimes also the pacemaker is moving back and forth along a preparation over a distance of two or more millimeters. These variations can easily be detected by placing the two leads close together in the region of the pacemaker. In such preparations, as would be expected, the size of the prepotentials fluctuates considerably.

Cardiac muscle is much less favorable for these studies than the ureter because the shortness of the intervals between beats makes it difficult to distinguish prepotentials from diphasic artifacts and after-potentials. The determination of the magnitude of the prepotentials in various regions gave convincing results only in about half the preparations used. In some preparations the potentials were too small to permit good comparisons. A more serious difficulty was found in the presence of an initial positive deflection in certain regions of the muscle, making the interpretation of the records uncertain. Furthermore, the prepotentials could not be detected in spontaneously beating preparations from the frog's ventricle nor in strips from the ventricle of the turtle in which activity had been induced by application of pure NaCl solution or Ringer's solution containing $BaCl_2$. These negative findings can be explained by assuming that in preparations with a low degree of automaticity the local potentials



Figs. 1–7

are restricted to small regions of the tissue or that they are weak and cause a discharge only if the excitability of the tissue is abnormally high.

More direct evidence for the existence of prepotentials was found in experiments where the muscle was depressed by injury or by acetylcholine. In the experiment illustrated in figure 2 a strip from the right auricle of the dog which had been dissected one day previously was used. It was at first quiescent and showed only slight and irregular electric activity. After a drop of adrenaline 1:100,000 was applied to one lead, the treated region began beating and potentials as shown in A were obtained. The discharge later ceased abruptly while slow rhythmic potentials continued (B). A comparison of these potentials with the prepotentials clearly shows that the gradual rise in negativity before each discharge is a phenomenon independent of the conducted impulses.

The local potentials can be separated from conducted impulses also by the application of drugs. Strips from the sinus venosus of the turtle and from the right auricle of mammals were irrigated with acetylcholine iodide 1:20,000 or β-methyl-acetylcholine (mecholyl) 1:400,000 and the recovery from the action of the drug was studied. All visible activity stopped but slow potential waves

Fig. 1. Prepotentials at pacemaker and at various distances from it. Preparation strip from sinus venosus of turtle. Below diagram of arrangement of the leads, *O*, origin of impulses, *X* location of leads in record *F*. Monophasic potentials obtained by S tube around right end of muscle. A: active lead at *O*. B: 2 mm, C: 4 mm. to right, D: 2 mm, E: 4 mm to left, F: diphasic. Time: seconds. Temp.: 22°.

Fig. 2. Potentials of a strip from the right auricle of the dog. Both leads on intact tissue. Activity was initiated by applying adrenaline $1:10^6$ to the region of one of the leads. In B sudden cessation of discharge with continuation of local potentials. Calibration 0.5 mVolt. Time: seconds. Temp.: 32°.

Fig. 3. Action of acetylcholine on the sinus venosus of the turtle. Monophasic. A, normal beat. B, after application of acetylcholine 1:20,000, only local potential at previous frequency. C, partial recovery, fully conducted impulse only every second local potential wave. The drug caused restoration of conduction inside glass tube, producing diphasic potentials. Time: $\frac{1}{5}$ second.

Fig. 4. Action of mecholyl on strip of sinus venosus of turtle. After A was recorded mecholyl 1:400,000 was applied. In B somewhat irregular, weak local potentials. C, D, E, stages of the recovery from the action of the drug. Note missed beats of pacemaker. Time: seconds.

Fig. 5. Oscillatory prepotentials from strip of sinus venosus of turtle produced by an excess of K ions. A, normal muscle. B, C, D, after irrigation with a mixture of Ringer's and 1 per cent KCl in the proportions 1:10, 2:10, 3:10 respectively. Without additional treatment in E further slowing of beat and increased number of oscillations, in F cessation of discharge while local oscillations persist. Temp.: 26°. Time: seconds.

Fig. 6. Luciani group from strip of right auricle of the cat showing oscillatory prepotentials. A, beginning, B, end of a group. Second beat missed as in all groups from this preparation. C, local oscillations without discharge shortly before D, final cessation of electric activity was recorded. In C and D amplification twice that in A and B. Temp.: 29°. Time: seconds.

Fig. 7. After-potentials of strip from turtle ventricle. Monophasic. Response elicited by electric shocks. A, Ringer's solution; B, after irrigation with isotonic NaCl solution; C, after irrigation with a mixture of equal parts of Ringer's and isotonic $CaCl_2$. Temp.: 22°. Time: seconds. Calibration: one mVolt.

were usually present. In many details the results were variable. In eight of the twenty preparations used the potential waves had the same frequency as the normal beat (fig. 3). Their size gradually increased until the discharge of impulses was resumed. At first the intervals between beats were long, as long as ten times the normal duration of a cycle, because the muscle missed some of the beats of the pacemaker. In the other preparations the activity of the pacemaker was at first rather irregular but returned to the previous frequency as soon as some impulses were discharged (fig. 4), or the frequency of impulses at first was definitely smaller than normal, though never less than half. It appears that an appreciable slowing of the rate by acetylcholine is chiefly caused by a failure of the muscle to respond regularly to the rhythmic local potentials and only to a minor extent by a slowing of the intrinsic rhythmicity.

The potentials during the recovery from the drug action often are very complex. This is, at least partly, due to the appearance of impulses which are conducted for only short distances (p. 14) and to some inco-ordination between different parts of the preparation. Sometimes another, independent, pacemaker comes into play temporarily as shown by the fact that the first conducted impulses are discharged without any relation to the beat of the previous pacemaker whose activity is being recorded.

2. *Oscillatory prepotentials.* In depressed cardiac muscle the impulses often are preceded by a series of potential oscillations of gradually increasing magnitude like those previously described for the ureter and other spontaneously discharging tissues (3, 6). Discharges always occur near a negative crest of one of the potential oscillations. The phenomenon could be produced by a variety of agents, by an excess of K or Ca ions or by mecholyl, or it occurred in preparations kept in the refrigerator overnight, but no treatment was found which invariably induced oscillatory prepotentials.

It is remarkable that, while the frequency of impulses may be slowed down many times, the intrinsic rhythmicity of the pacemaker can be modified only moderately. Thus in figure 5 moderate amounts of K-ions slowed down the intervals between beats (B), but as the intervals became longer (C) potential waves appeared between the beats at a frequency close to that of the normal beat and these rhythmic variations continued at high K ion concentrations with unchanged frequency while no impulses were discharged and until all signs of activity had disappeared (D).

In preparations with oscillatory prepotentials impulses often are discharged in groups because several waves in succession initiate an impulse. In the preparation from which figure 6 was obtained, 14 almost identical groups, each consisting of 40 to 60 beats, were recorded. During the intervening period of about 2 minutes no rhythmic activity could be detected. At the end of the series there were groups of oscillations without any discharge (fig. 6C). The rhythmicity of the muscle remained perfectly regular until all signs of spontaneous activity stopped (D). The grouping of the impulses into bursts has previously been described under the name of "Luciani periods" and was observed under a variety of conditions injurious to the muscle (cf. 11). An analogous phenom-

enon, also characterized by oscillatory prepotentials, was found in the ureter under similar conditions.

B. *After-potentials.* The after-potentials of cardiac muscle will be described here because, like the prepotentials, they may initiate impulses and are important for the understanding of certain rhythmic phenomena.

The possibility of a diphasic artifact makes the interpretation of these potentials uncertain during the first second, but the later phases undoubtedly represent a separate phenomenon.

In quiescent, fresh strips from the turtle's heart each response is followed by a weak positive after-potential lasting for about 10 seconds. It is increased after a short series of responses (fig. 7) but after prolonged stimulation it becomes smaller again or it may even be replaced by a negative after-potential. The positive after-potential is furthermore increased by irrigation with calcium free Ringer's solution or isotonic NaCl solution (fig. 7B). After repeated irrigations the preparations often beat spontaneously at a fairly rapid rate so that the after-

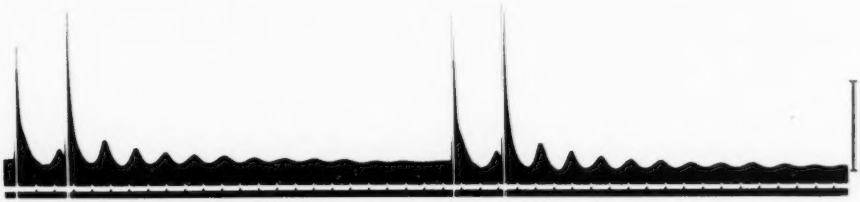


Fig. 8. Oscillatory after-potentials from a strip of the right auricle of the rabbit produced by application of a mixture of equal parts of Ringer's and isotonic $CaCl_2$. Monophasic. Groups of two impulses, discharged independently of the oscillations. Recorded with oscillograph. Time: $\frac{1}{5}$ second. Temp.: 34°. Calibration: 0.5 mVolt.

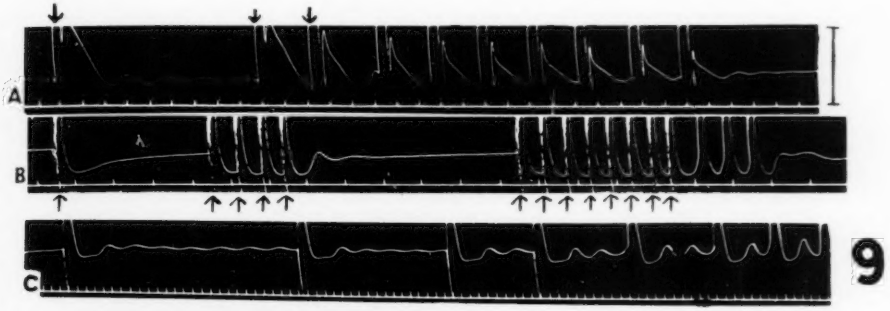
potentials cannot be studied. In preparations in which this does not occur the positive after-potential gradually becomes smaller again and finally a single slow negative wave appears instead.

In analogy with nerve, one might expect that the positive after-potential is associated with subnormality and it is particularly suggestive to assume that its presence causes the inhibition which follows a series of extrasystoles in the auricle as well as ventricle (Erlanger and Hirschfelder, 8; for later work, cf. 11). However, in preliminary observations no correlation between the duration of the positive after-potential and the duration of the inhibition could be observed.

Muscles which are studied one day after dissection often show oscillatory after-potentials consisting of waves of gradually decreasing magnitude resembling damped oscillations. This phenomenon can be produced most easily in strips from the turtle heart by treatment with a solution containing a large excess of Ca-ions (fig. 7C). The oscillations are present only in the region treated with the solution. Following a short series of responses they are larger than after a single discharge, but the amplitude declines after more than about ten responses. An excess of calcium ions sometimes produces this type of after-

potential also in preparations from mammalian hearts (fig. 8). The effects of the solutions used are completely reversible.

Adrenaline added to the solutions ($1:10^6$) increases, usually about doubles, the magnitude of the oscillations in the auricle and ventricle. Acetylcholine



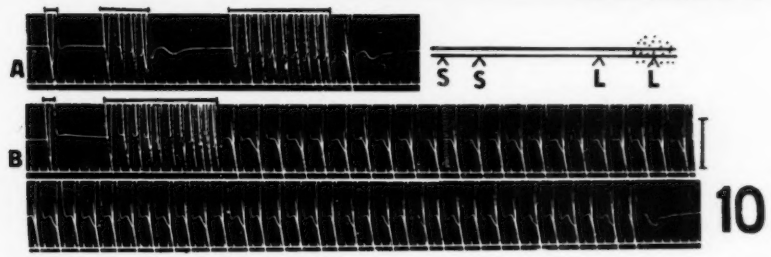


Fig. 9. After-discharge produced by oscillatory after-potentials. Stimuli are indicated by arrows. All the other impulses induced by oscillations. A, strip from turtle ventricle treated with equal parts of Ringer's and isotonic $CaCl_2$. Temp.: 22°. Calibration one mVolt. B, strip from right auricle of dog 24 hours after dissection. Monophasic. Temp.: 32°. C, shift in the origin of impulses as a result of the appearance of oscillations. Strip from the sinus venosus of the turtle treated with mixture of 2 parts isotonic $CaCl_2$ and one part Ringer's. All impulses discharged spontaneously. Discharge ceased about 3 minutes later. After a period of rest, during which oscillations almost disappeared, the same sequence was recorded three times. Temp.: 19°. Time: seconds.

Fig. 10. After-discharge produced by oscillatory after-potentials, showing that it originates in region with oscillations. Strip from ventricle of turtle. Diphasic potentials. Arrangement of electrodes shown in diagram. S, stimulating electrodes; L, leads for amplifier. Region of right lead was treated with a mixture of equal parts of Ringer's and isotonic $CaCl_2$. The initial impulses (indicated by horizontal line above), beginning with an upstroke, were elicited by electric shocks. The impulses of the after-discharge begin with a slow down-stroke which is the first wave of the after-potential. In the upper record the after-discharge had only one impulse, in the lower record from the same preparation, a longer period of stimulation was followed by an unusually long after-discharge. Calibration: 1 mVolt. Time: seconds.

and mecholyl strongly depress the oscillations in the auricle but have no effect on the ventricle, an observation which is in line with the absence of other inhibitory effects of acetylcholine on the ventricle. It is remarkable that the

drugs studied may increase or decrease the magnitude of the oscillations but do not change their frequency.

The oscillatory after-potentials are particularly interesting because they may cause the discharge of impulses. In preparations with oscillatory after-potentials a short series of responses, rarely also a single response, may be followed by additional impulses which I shall call the after-discharge (fig. 9A, B). These impulses arise from the negative phases of the after-potential and are fired off if the latter reach threshold value. The tendency to give after-discharge is greatest under conditions where the after-potentials are largest, following a short series of responses and under the influence of adrenaline.

That the impulses of the after-discharge do not originate at the stimulating electrode is shown in the experiment illustrated in figure 10. Diphasic potentials were recorded and the region of the distal lead alone was treated with a solution containing an excess of calcium ions. The impulses of the after-discharge were conducted back toward the stimulating electrodes and evidently originated in the region treated with the solution. The after-discharge usually consists of one or a few impulses but may also last for several minutes. The termination of the after-discharge can be explained by the gradual decrease in the size of the oscillations during a long series of responses.

The presence of oscillatory after-potentials may lead to peculiar rhythmic phenomena. In the experiment illustrated in figure 9C a preparation from the sinus venosus of the turtle was treated with a large excess of calcium ions. The beat became intermittent and each series of impulses was introduced by an accelerating series of beats originating close to the distal lead. The oscillations induced by these spontaneous beats increased after each discharge until they were so large that they determined the rhythm of the beat and the pacemaker shifted suddenly to the region of the first lead.

After-discharge consisting of one single impulse had previously been observed by Schmitt and Erlanger (12) who explained the phenomenon as due to the reentry of an impulse and called it opisthodromic conduction. Ashman and Hafkesbring (5) observed after-discharges of longer duration and attributed them to the presence of a phase of supernormal excitability.

Recently Harris and Moe (19) described rapid oscillations produced by constant current of high intensity, which they consider analogous to the oscillations observed by Arvanitaki in nerve fibers. However, the conditions under which the observations were made and the high frequency of the oscillations make it doubtful that they are similar to the decrementing and incrementing local potentials described here.

Discussion. It appears that there are two types of rhythmic local potentials as had previously been observed in the ureter. In the first type, which is responsible for the initiation of the normal beat and can most conveniently be studied after the application of acetylcholine, repolarization follows the phase of depolarization rather abruptly, and the potential waves sometimes continue for appreciable periods without great changes in magnitude (fig. 2B, 3B). Their frequency is influenced, in some cases, by acetylcholine and by adrenaline,

and it varies considerably between different preparations. The second type, found only in injured muscle, is distinguished by its great regularity and it resembles in its time relations oscillations of simple physical systems. Their magnitude usually increases and decreases in a regular manner and, in the turtle heart, their frequency is surprisingly constant and unaffected by acetylcholine, adrenaline, or even by extreme variations of the ionic composition of the medium. How the first type of potentials may change over into the second type is illustrated in figure 5.

The ascent and descent of the potential waves, as long as they are weak, are usually roughly symmetrical. However, as their magnitude approaches threshold the ascent becomes progressively steeper as compared with the descent. This effect may be due to an additional non-propagated response which is, perhaps, analogous to the local response in non-medullated nerve fibers produced by electric shocks of near threshold strength (10). In fully excitable muscles the propagated impulses arise quite abruptly from the local response. However, in muscles which have been injured or treated with high concentrations of acetylcholine responses of many different sizes, intermediate between local responses and fully conducted impulses, may be found. Probably these responses are caused by impulses which are conducted only for short distances, and, therefore, are comparable to the responses of depressed nerve fibers where likewise intermediate stages between local responses and fully conducted impulses occur (10).

That there is no essential difference between the oscillatory pre- and after-potentials is suggested by the observation that both can initiate impulses and by the fact that their frequency is the same. Furthermore, in preparations in which the prepotentials are oscillatory the after-potentials have the same character and, in fact, one may consider the after-discharge as a Luciani period induced by electric stimulation. The difference between preparations showing this phenomenon, like strips from the ventricle of the turtle and muscles which often give spontaneous Luciani periods, for example the sinus venosus, lies merely in the different degree of automaticity.

The magnitude of the oscillations of the after-potential may diminish rapidly so that only one or two waves can be seen or they may decrease so slowly that as many as thirty oscillations can be counted. To describe these differences one may speak of different degrees of damping (cf. 3, 4). It is significant that the damping is smallest in preparations with automaticity (fig. 8, 9C), like those of the sinus venosus, and largest in preparations which never discharge spontaneously, like those from the turtle's ventricle. Prepotentials can also be considered in this connection and they can be characterized by negative damping. There appear to be all transitions between these potentials and the highly damped after-potentials of ventricular muscle.

It is apparent that many rhythmic phenomena in cardiac muscle cannot be understood if only conducted impulses are recorded. It is quite possible, therefore, that the local, graded activity which may take place in the "resting" state offers the key to the understanding of a variety of disturbances of the rhyth-

micity of the heart. The oscillatory after-potentials, for instance, provide a simple explanation for coupled extrasystoles and paroxysmal tachycardia. Unfortunately the study of these weak and localized potentials in the heart in situ presents great technical difficulties.

SUMMARY

The action potentials of isolated cardiac muscle were recorded in an attempt to detect the processes which initiate the beats and which are responsible for the rhythmicity of heart action. Confirming earlier work on other muscles with automaticity it was found that spontaneous impulses are initiated by weak local potentials which are present in a large part of the muscular tissue but which are strongest near the origin of the impulses. A phase of gradually rising negativity precedes each impulse in muscles with normal rhythmicity, but in injured muscles there may be instead regular potential oscillations of gradually increasing magnitude. These oscillations give rise to the phenomenon of the Luciani periods if several waves in succession cause the discharge of impulses.

Each impulse is followed by an after-potential which is normally positive but which is oscillatory under certain abnormal conditions. The oscillations of the after-potential may give rise to the discharge of further impulses like those of the prepotentials. Acetylcholine diminishes the magnitude of the oscillations in the auricle and sinus venosus, but not in the ventricle; adrenaline increases their magnitude. None of the drugs alters their frequency.

It may be expected that a consideration of the local potentials will be helpful in the understanding of certain types of abnormal rhythmicity of the heart.

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THE ELECTRICAL ACTIVITY OF A THALAMOCORTICAL RELAY SYSTEM

E. W. DEMPSEY AND R. S. MORISON

From the Department of Anatomy, Harvard Medical School, Boston, Massachusetts

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From time to time in the course of experiments dealing with cerebral pathways and thalamocortical relationships, it was observed that single stimuli applied to peripheral nerves or to ascending sensory pathways produced a repetitive series of cortical potentials in addition to the usual sensory response (Morison, Dempsey and Morison, 1941a), and that these repetitive potentials were sharply localized within the cortical area receiving sensory projection fibers (cf. Adrian, 1941). The "repetitive sensory response" was grossly similar, except for its localization, to the spontaneous bursts of cortical activity characteristic of nembutal anesthesia and to the bursts of activity set up by the recruitment response (Dempsey and Morison, 1942a). Because of these similarities, it was frequently difficult to determine whether the response to a given stimulus resulted from activity in medial thalamic mechanisms (Morison and Dempsey, 1942) or to activity in other circuits whose localization was unknown. Similarly, repetitive stimulation frequently brought about a successive increase in the evoked potentials reminiscent of the recruiting response consequent on stimulation of intralaminar thalamic structures (Dempsey and Morison, 1942a). Striking differences in latency and anatomical localization, however, suggested that an attempt to separate these various phenomena one from another by as many criteria as possible might illuminate the problem of thalamocortical interaction and consequently facilitate the analysis of spontaneous cortical activity. It was therefore decided to extend these investigations in order to determine the characteristics of the various effects for comparison with those of other cerebral systems.

MATERIAL AND METHODS. Cats, anesthetized with nembutal (0.65 cc./kgm), were used. Condenser discharge stimuli were led to the animal through a transformer and a Wagner ground for controlling the shock artifact. Cortical responses were picked up by bipolar silver or steel electrodes applied either on the surface of the pia or from the pia to a deep electrode inserted about 2 mm. into the cortical substance. The usual procedure was to explore the cortex with the surface bipolar electrodes until the point yielding maximal potentials to peripheral nerve stimulation was reached. At this point the deep electrode was inserted into the cortex, and subsequent observations were made so that an upward deflection signified negativity at the surface electrode. Bipolar steel wires, insulated except at the tips and having a separation distance of approximately one-half millimeter, were inserted into the brain by means of a stereotaxic instrument (Morison, Dempsey and Morison, 1941b) and utilized either for recording or stimulating. After suitable amplification, the potentials were re-

corded by a 5-channel Grass inkwriter, a DuBois oscillograph or a 2-channel cathode ray oscillograph.

At the end of each experiment the brain was removed and fixed in 10 per cent formalin. Serial frozen sections were prepared (Marshall, 1940) and stained with thionin. A direct positive print was prepared by projecting at 10 times magnification the sections containing the needle tracks made by the stimulating and recording electrodes. A permanent record was therefore provided of the electrode position corresponding to each electrical record taken during the experiment.

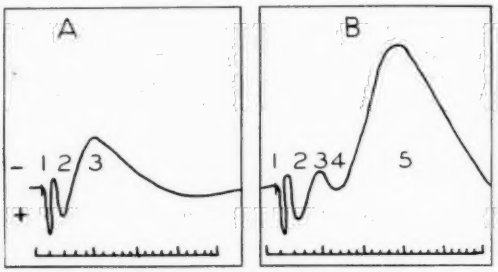

Fig. 1

Fig. 2

Fig. 1. Tracings of cortical responses to stimulation of the internal capsule. The potentials were recorded by surface to deep electrodes, one pole on the pial surface and one inserted 2 mm. into the cortical substance. The electrodes were connected so that an upward deflection signifies negativity at the surface electrode. Time scale, 1 + 5 msec.

A. Response to single shock. Three components are present, the first two being positive and the third a negative deflection. This complex is referred to in the present paper as the "primary response."

B. The fourth response to a series of stimuli at a frequency of 8 per second. A fourth, positive and a fifth, negative deflection have appeared following the original three components. Components 4 and 5 increase dramatically in size during repetitive stimulation, and are referred to as the "augmenting response."

Fig. 2. Separation of thalamic regions yielding primary and augmenting responses.

A. Stimulus to medial part of *n. ventralis lateralis pars externa*. Pure augmenting effects are shown in the cortex.

B. Stimulus 1 mm. lateral to the position from which record A was obtained. Both primary and augmenting effects are present. Time signal, 250 cps.

RESULTS. A. *Description of responses.* When single shocks were applied to the ventrolateral thalamus, cortical responses occurred in which three component deflections could be recognized: 1, a surface positive, spike-like deflection with a latency so short that it frequently merged with the stimulus artifact; 2, a positive deflection of 0.5 to 0.8 msec. latency which was followed by 3, a prolonged negative deflection.

Application of a short train of stimuli at a suitable frequency caused the appearance of two further components. These were: 4, a positive deflection whose latency was approximately the same as the negative peak of the third element; and 5, a second negative element. Figure 1 indicates diagrammatically the typical sequence, latency and duration of these components.

The diverse components of the wave complex reacted differently in various experimental conditions. The first element followed higher frequencies of stimulation than any of the others, and, although reduced in size, was still recognizable at stimulus frequencies of 400 per second. Next most resistant to high frequencies was the second component which remained at stimulus frequencies of around 100 per second. The third element usually was severely reduced at frequencies of 30 to 50 per second.

Stimulation with lower frequencies (optimal at 8–15 per sec.) was followed by the progressive development, after the first shock, of the 4th and 5th components (fig. 3, A and B). This progressive enhancement is a striking characteristic and has suggested the term "augmenting response." The first three components, on the other hand, showed no augmentation but only reduction on repetitive stimulation. They are referred to in the present paper as the "primary response" (fig. 3, C and D).

In favorable preparations, the primary potential, elicited by a single shock, was followed by a train of waves the frequency of which was similar to that of spontaneous bursts which ordinarily constitute the predominant cortical activity of the nembutalized cat (fig. 7A). This "repetitive sensory response" was not seen invariably (cf. Adrian, 1941), nor have the precise conditions necessary for its production been identified, though light anesthesia may be said to favor its occurrence.

B. *Anatomical localization.* The cortical area from which the augmented response could be recorded varied with the placement of the stimulating electrodes. Shocks just above threshold strength ordinarily produced primary responses localized to the primary divisions of the sensory cortex and augmented responses localized to these and the corresponding areas anterior and posterior. These observations indicate that whenever primary and augmented responses were both provoked, the cortical area yielding augmented responses was greater than that from which the primary response could be recorded.

In several experiments observations were made which suggest an anatomical separation of the systems responsible for the two types of effects. Thus in certain positions of the stimulating electrodes only primary responses were produced, while in others pure augmenting potentials were obtained (fig. 2A). In either location, application of stimuli of greater intensity was followed by the total wave complex, i.e., by both primary and augmenting responses (fig. 2B).

Results identical with those produced by thalamic stimulation were obtained, when the stimulating electrodes were placed in the internal capsule. Here, as from thalamic activation, primary responses were localized to smaller cortical regions than were the augmenting effects, and capsular areas yielding only primary or augmenting responses were found.

Stimulation of the medial lemniscus and sensory nerves with repetitive volleys also was followed by effects attributable to augmentation. The sensory nerve effects were less striking than were those of the lemniscus, thalamic nucleus and internal capsule. Nevertheless, in favorable preparations nerve activation produced clear augmenting responses. In the majority of the experiments, how-

ever, the voltage of the sensory potential complex did not increase appreciably, but changes in the wave form of the response occurred. The positive initial deflection broadened and the negative response was delayed (fig. 4). These changes have been interpreted as an addition of the augmenting effect to the shorter latency true primary response.

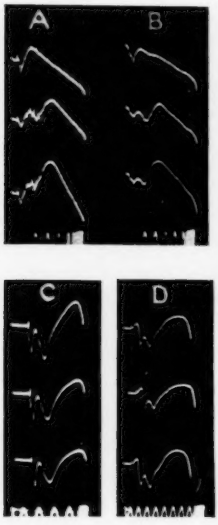

Fig. 3

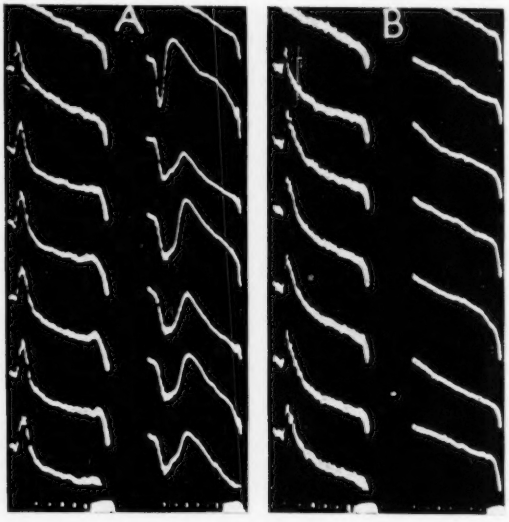

Fig. 4

Fig. 3. Primary and augmenting responses induced by sensory stimulation.

A. Surface to deep recording electrodes. Stimulation 8/sec. to internal capsule. Time signal, 200 cps. Both primary and augmenting responses are present. Compare with figure 1.

B. Same as A, but surface bipolar electrodes.

C. Stimulus to another position in the internal capsule. Only the primary response is present. Compare wave form with figure 1A. Time signal, 500 cps.

D. Stimulus to medial lemniscus. The wave form is similar to that seen after capsular stimulation, but the latency is longer. Time signal, 500 cps.

Fig. 4. Augmentation in thalamus and cortex on repeated stimulation of a sensory nerve. Simultaneous records: left, from *n. ventralis lateralis pars externa;* right, from radial cortical projection. Stimuli to radial nerve at 8 per second.

A. Note broadening of thalamic response. The duration of the positive cortical wave increases and the negative peak is shifted to the right. Time signals, 100 cps.

B. Same after decortication. The broadening of the thalamic response is not abolished. Time signals, 100 cps.

The repetitive sensory response was confined quite sharply to the area of the sensory cortex yielding primary potentials (cf. Adrian, 1941). In addition to its appearance in the sensory cortex, the repetitive response was recorded from electrodes placed in *n. ventralis lateralis pars externa* of the thalamus (fig. 5) and from the internal capsule. Repetitive potentials did not occur, however,

when recording electrodes were inserted into the medial lemniscus posterior to the lateral thalamic nuclei.

The repetitive cortical response has been evoked by single stimuli applied anywhere along the ascending sensory system. Indeed, it was more easily elicited by stimuli delivered to the medial lemniscus or thalamic nuclei than by activation of the peripheral nerve. It was frequently observed that preparations whose response to nerve stimulation was poor yielded excellent responses when stimulated either at the level of the thalamic synapse or the thalamic radiations.

It is apparent from a consideration of the characteristics of the primary response (elements 1, 2 and 3) and the augmented response (elements 4 and 5) that they react in various respects as two independent units. The differences in

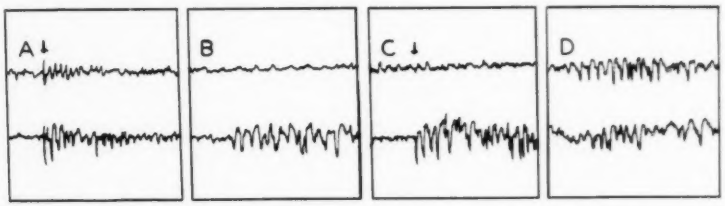

Fig. 5. Localization in thalamus of repetitive sensory response and burst activity. Upper records from thalamus; lower records from radial cortical projection. Arrows signal a single stimulus to the radial nerve. Paper speed 7.5 mm. per sec.

A. Thalamic electrodes in *n. ventralis lateralis pars externa*. Note immediate response to radial stimulus, followed by repetitive waves in cortex and thalamus.

B. Same recording position. The record is of a spontaneous cortical burst. Note absence of thalamic activity.

C. Thalamic electrodes 2 mm. medial and 1 mm. above the position from which records A and B were obtained. The immediate and repetitive sensory responses are not obtainable at this location.

D. Same recording position. Note activity in thalamus correlated with the spontaneous cortical burst.

behavior of these two units may be summarized as follows. 1. The augmenting response ordinarily requires previous facilitation for its appearance, while the primary response appears full sized after a single stimulus. 2. The primary response always has a shorter latency than that of the augmenting response. Consequently, capsular or thalamic stimulation results in clearly separable effects, while after sensory nerve stimulation the greater opportunities for temporal dispersion result in the augmenting response appearing as a distortion of the terminal phase of the primary response. 3. The primary response is localized to a smaller area in the cortex and thalamus than is the augmenting response, and for any given stimulus, areas can be found from which each response is recordable without the other.

C. *Interactions of thalamocortical potentials.* 1. *Repetitive, augmenting and primary sensory responses.* The various considerations outlined above indicate that the augmenting response represents a state of physiological activity different

from that indicated by the primary response. The alternative view, that mere recruitment of similar but slower elements constitutes the difference between them, would imply that no qualitative differences in behavior should occur. Consequently, experiments were performed in which the repetitive response was induced by application of a single shock delivered variously to the peripheral nerve, thalamic nucleus or thalamic radiations. At varying intervals after this first shock a second stimulus was applied to the peripheral nerve so that the

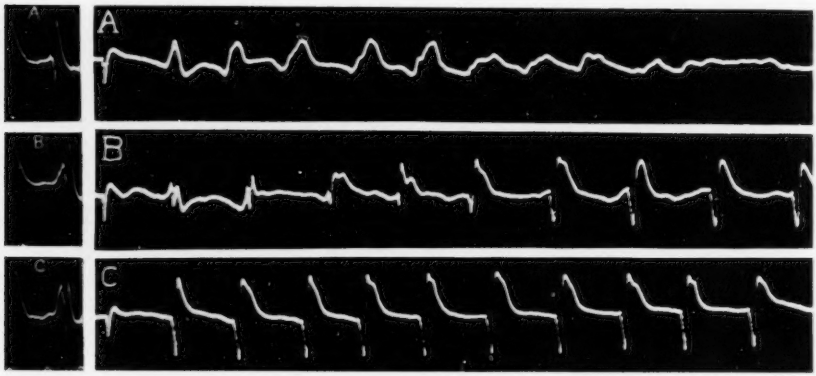

Fig. 6 Fig. 7

Fig. 6. Superimposition of the sensory primary response upon the repetitive potential. Two shocks were delivered to the radial nerve timed so that the second fell upon the repetitive potential at varying phases. Record from radial cortical projection, surface to deep electrodes. Time signal, 10 cps.

Fig. 7. Interaction of the repetitive response with the augmenting sensory response. Radial cortical projection, surface to deep electrodes. Stimuli to the ventrolateral thalamic nucleus.

A. Single shock, showing immediate and repetitive responses. Note that the first five repetitive potentials are nearly equal and are followed by 3 or 4 smaller effects suggesting a stepwise gradation in size.

B. Stimuli at a frequency of 7 per sec. At this rate each succeeding stimulus falls upon the repetitive potentials, and augmentation does not occur until the repetitive effect is fatigued.

C. Stimuli at a frequency of 8 per sec. Each stimulus precedes the repetitive effect and immediate augmentation is produced.

primary response fell at varying phases upon the individual potentials of the repetitive response. The primary response induced by the second test shock was not blocked at any phase of the repetitive response (fig. 6). On the other hand, evidence was supplied by experiments such as that illustrated in figure 7B which shows that succeeding shocks when timed to fall during a repetitive potential failed to evoke augmented responses. Slightly shorter intervals between stimuli resulted at once in good augmentation but obliterated the repetitive response (fig. 7C). The conclusion seems inescapable that the augmenting and primary responses represent activity in separate neuronal systems since they interact dif-

erently with the repetitive effect. Similarly, the obliteration of either the augmenting by the repetitive potential or *vice versa* strongly suggests that they represent different states of activity in the same neuronal system.

2. *Repetitive sensory response and spontaneous activity.* The similarity in wave form and general appearance of the repetitive response and the generalized 8–12 per sec. spontaneous potentials which characterize nembutal anesthesia prompted an investigation of their interactions. The repetitive response was evoked at various phases of the spontaneous bursts. Stimuli delivered during a burst and timed so as to throw into phase the repetitive and spontaneous rhythms enhanced the potentials immediately following the shock. Moreover, when the

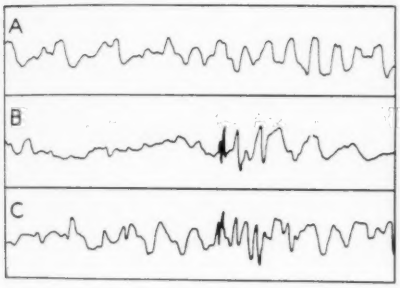

Fig. 8

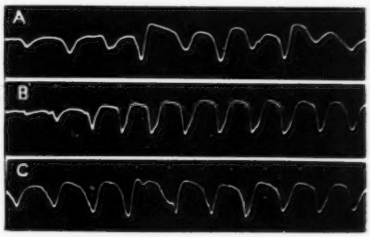

Fig. 9

Fig. 8. Interaction of repetitive and spontaneous burst effects. Radial cortical projection, surface to deep electrodes. Paper speed, 30 mm. per sec.

A. Spontaneous burst.

B. Immediate and repetitive effect of a single radial nerve stimulus.

C. The repetitive effect induced out of phase with the spontaneous potentials. Note the doubling of the cortical frequency.

Fig. 9. Superimposition of augmenting sensory response upon spontaneous burst potentials. Radial cortical projection. Surface to deep electrodes. Stimuli at a frequency of 10 per sec. signaled by dots.

A. Spontaneous burst.

B. Augmenting responses induced during an interval between bursts.

C. Augmenting responses induced during a burst of spontaneous potentials. Note the appearance of augmented responses when evoked during the spontaneous effects.

repetitive response was induced out of phase with the spontaneous burst, the individual repetitive potentials were interpolated between the spontaneous waves in such a way that the cortical rhythm suddenly doubled in frequency (fig. 8). Consequently, since two forms of activity can not simultaneously occupy a common pathway, it follows that the neuronal elements responsible for the burst and the repetitive effects are different.

Spontaneous activity other than the 8–12 per sec. rhythmic bursts can also be distinguished in the electrocorticogram. Varying amounts of random activity existed during the intervals between bursts (cf. Derbyshire, Rempel, Forbes and Lambert, 1936). This activity was more consistently recorded from electrodes placed upon the posterior sigmoid, marginal and ectosylvian gyri than from

other cortical areas. This characteristic has prompted the tentative name of "projection" activity which will be used throughout this paper.

The amount of this background projection activity ordinarily varied directly with the level of anesthesia, although in a few preparations large amounts of nembutal were required to suppress it. In such preparations, however, the activity was greatly reduced by decerebration (fig. 10). This fact indicates a difference between the projection and burst activities, since the latter was not depressed by section of the brain stem (fig. 10B). However, section of the thalamic radiations or removal of the entire thalamus abolished both projection and burst activity, leaving only the widely separated waves which have been associated with an extrathalamic system (Morison, Dempsey and Morison, 1941b).

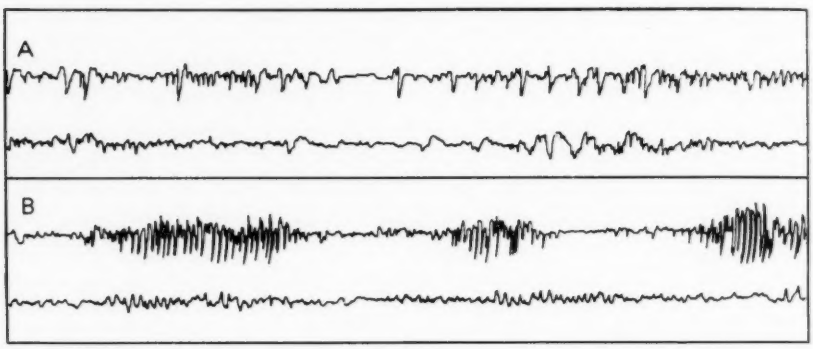

Fig. 10. Effect of decerebration upon spontaneous "projection" and "burst" activity. Upper record, radial cortical projection. Lower record, middle suprasylvian gyrus. Paper speed, 7.5 mm. per sec.

A. Irregular activity characterized as "projection" activity, upon which bursts are superimposed.

B. After intercollicular section. Note the decrease in amount of irregular "projection" activity and improvement in bursts.

Projection activity in the cortex, when present, was correlated with activity of similar frequency and appearance in the ventrolateral thalamic nucleus. Figure 11 shows simultaneous records from thalamus and cortex from a preparation in which projection activity was a noteworthy feature. The amount of thalamic activity greatly exceeded that recorded from the same thalamic region in animals whose prevailing cortical activity was of the burst variety (fig. 5B).

Because the individual potentials of the projection activity did not repeat themselves at regular intervals, it was difficult to study interaction with other effects. Nevertheless, certain statements can be made. The repetitive response was produced ordinarily in preparations whose depth of anesthesia was only slightly greater than that necessary to depress the projection activity. The addition of more nembutal to such preparations abolished or reduced the repetitive activity. Furthermore, in occasional instances when the projection ac-

tivity did not disappear after deeper narcosis was induced, good repetitive sensory responses did not occur. The usual effect was the production of one or two potentials following the stimulus at intervals comparable to those of the repetitive response, but later events were obscured by the simultaneous presence of the projection activity.

3. *Repetitive sensory response and the recruiting response.* Stimulation of medial thalamic structures associated with the internal medullary lamina has been shown to produce long latency recruiting potentials which have many characteristics in common with the spontaneous 8–12 per sec. bursts (Dempsey and Morison, 1942a). The interaction of these potentials with the repetitive response was studied. Figure 12 shows that recruiting responses in no way interfered with the repetitive responses. Similarly, the inhibition of spontaneous activity by high frequency stimulation of recruiting areas did not affect the

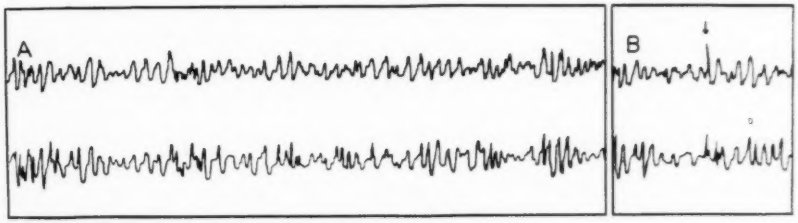

Fig. 11. Simultaneous presence of projection activity in cortex and thalamus. Upper record, *n. ventralis lateralis pars externa*. Lower record, radial cortical projection. Paper speed 7.5 mm. per sec.

A. Spontaneous activity. Note similarity of thalamic and cortical records. Compare with figure 1B for similar recording position when projection activity was absent.

B. Responses to single shock to radial nerve, applied at arrow.

repetitive response (fig. 12D), although such stimulation prevents the appearance of the spontaneous bursts (Dempsey and Morison 1942a).

4. *The augmented sensory response and spontaneous activity.* Application of a series of stimuli to the ventrolateral thalamus or radiations during a burst of spontaneous potentials led to augmentation of the cortical response regardless of the phase of spontaneous activity (fig. 9).

Less regularity has been encountered when augmentation was induced in the presence of projection activity. Application of a single shock anywhere in the sensory system ordinarily led to the appearance of only the "primary" potential in the cortical area while to evoke the augmented response repeated stimuli were required. Nevertheless, when single stimuli were applied to the lemniscus or capsule in preparations showing spontaneous projection activity, responses were produced, one component of which had a latency corresponding to element 4. The voltage of this effect varied depending upon the preceding activity. During and immediately after a spontaneous projection potential only the short latency phenomena (elements 1, 2 and 3) were seen. At longer intervals, responses at the latency of element 4 appeared. These effects sometimes were so large as to

overshadow the true primary response. In such preparations, stimulation of the nerve or sensory tract at a slow frequency (1 per 2 or 3 sec.) was followed by responses whose voltage varied depending upon the phase of projection activity at which the stimulus fell.

When two shocks were applied two or three hundred milliseconds apart, there was augmentation of the second response only when it fell at a time when no projection potential was present, although augmentation always occurred at these long intervals when the projection and repetitive effects were absent (cf. Morison and Dempsey, 1943).

Application of a series of shocks at slow frequencies to preparations in which projection activity was present produced no augmentation when the shock fell

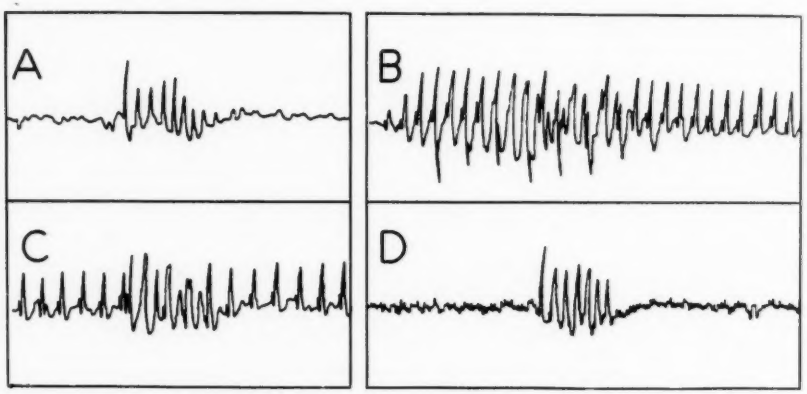

Fig. 12. Interaction of repetitive effect and recruiting potentials induced by stimulation of intralaminar thalamic regions (cf. Dempsey and Morison, 1942a). Radial cortical projection. Surface bipolar recording. Paper speed, 15 mm. per sec.

A. Immediate and repetitive potentials induced by a single shock to the radial nerve.

B. Recruiting effect of intralaminar thalamic stimulation. This effect rapidly equilibrates.

C. Repetitive response induced during equilibrated recruiting effect.

D. Repetitive response induced during 60 per sec. stimulation of the recruiting area. This high frequency stimulation abolishes burst activity (cf. Dempsey and Morison, 1942a).

upon a potential already existing. Stimulation at a rate of 7 per sec. usually led to block, an effect reminiscent of the superimposition of stimuli on the repetitive response. Faster frequencies, 8 to 60 per sec., invariably caused augmentation. Moreover, at these faster frequencies the projection activity was suppressed during the stimulation and occurred again upon its cessation.

Discussion. The possibility that certain components of the electrical activity of the brain may be separated, one from another, and studied as unitary phenomena has been emphasized previously (Morison and Dempsey, 1942). In the present paper, the possibility of separating spontaneous bursts from repetitive sensory responses has received especial attention because of their general similarity in form and sequence.

Several lines of evidence exist which reveal that the resemblances between spontaneous bursts and repetitive effects are only superficial. The conclusion that they represent states of activity in completely independent neuronal circuits may be summarized in the following manner. 1. The repetitive and burst potentials may be simultaneously evoked, an effect impossible if they utilized a common pathway (Sherrington, 1906; Dempsey and Morison, 1942b). 2. The cortical distribution of the two phenomena is different (p. 286). 3. The thalamic distribution of the two effects is separate (fig. 2). 4. Stimuli which modify burst activity do not affect the repetitive response (fig. 12). 5. The augmented response is blocked by repetitive potentials (fig. 7B), but not by burst potentials (fig. 9). 6. Eserine and acetylcholine markedly augment the repetitive responses while leaving the spontaneous bursts relatively unaffected (Chatfield and Dempsey, 1942; Morison and Dempsey, 1943). 7. The rhythm of the repetitive response is controlled by the delivery of the last shock of a series (Morison and Dempsey, 1943) while the rhythm of the spontaneous burst is not retimed by subsequent stimulation (Dempsey and Morison, 1942a). These considerations indicate, each with varying degrees of certainty, that spontaneous burst and repetitive responses reflect activity in totally different neuronal circuits. Taken together, their internal consistency enormously increases the probability of the conclusion.

There is also evidence that the neuron systems which mediate the repetitive and augmenting sensory responses can contribute an element to the so-called spontaneous activity of the cortex. Attention has been directed in the present paper to a type of activity that occurs in lightly anesthetized preparations to which the name "projection" activity has been applied. This type of activity is most dramatically revealed in the cortical areas upon which the great sensory systems project. It is ordinarily quite sensitive to anesthesia. Sections of the great afferent pathways abolish or greatly depress it (fig. 10). There are certain interactions between it and sensory responses which indicate neuronal linkages and common paths. Its presence in the cortex is correlated with activity of similar temporal characteristics in the ventrolateral thalamus (fig. 11). Conversely, absence in the cortex is accompanied by a reduction in the thalamic activity (fig. 5).

The characteristics of the projection activity listed above serve to distinguish it from the spontaneous 8 to 12 per sec. bursts which have been described previously (Derbyshire, Rempel, Forbes and Lambert, 1936; Dempsey and Morison, 1942a). The rhythmic bursts are best recorded from association cortical regions. They are quite resistant to anesthesia. Section of the afferent pathways never abolishes, but frequently enhances the rhythmic bursts (fig. 10). The various sensory responses can be superimposed with no suggestion of block upon the burst potentials. Their presence in the cortex is correlated with activity in medial rather than lateral thalamic structures (fig. 5; Morison and Finley, unpublished data). Lastly, stimuli which inhibit the spontaneous bursts either do not affect or actually enhance the projection activity (Morison and Finley, loc. cit.).

The data here presented permit the resolution of several difficulties which have obscured the mechanisms utilized in spontaneous activity and reconcile certain apparently contradictory statements in the literature. There is general agreement that section of the thalamocortical radiations abolishes or greatly depresses the spontaneous cortical waves (Bishop, 1936; Dusser de Barenne and McCulloch, 1938; Lewy and Gammon, 1940). Nevertheless, certain types of activity still persist after such cortical deafferentation (cf. Bishop, 1936; Bremer, 1938; Morison, Dempsey and Morison, 1941b). When sections of the afferent paths are made at lower levels, however, there is less agreement. Dusser de Barenne and McCulloch (1938) and Bremer (1938) have reported a persistence of spontaneous cortical activity after intercollicular section, while Lewy and Gammon (1940) report that with nembutal, but not with ether anesthesia, section of the afferent paths in the medulla or pons depresses and in the midbrain abolishes spontaneous potentials. Intermediate in position between these contradictory findings is the report by Dubner and Gerard (1939) that spontaneous potentials in the lateral geniculate bodies and optic cortex remain for one to two hours after deafferentation or maintenance in the dark, but can be reinitiated in the latter preparation by photic stimulation.

Similar confusion has attended attempts to interpret evoked potentials in thalamocortical circuits. Bishop (1936) and Bartley (1942) found that the visual cortex response to optic stimulation varied depending upon the phase of spontaneous activity present at the time. Moreover, on the basis of local strychninization of the somasthetic cortex and the ventrolateral thalamic nucleus, Dusser de Barenne and McCulloch (1938) suggested that a closed chain circuit existed between cortex and thalamus, and that spontaneous activity resulted from activity within this self-re-exciting system. Such a system could easily account for Bishop's and Bartley's observations that afferent impulses were blocked and facilitated periodically by spontaneous potentials. On the other hand, it is difficult to reconcile such a system with Marshall, Woolsey and Bard's report (1941) that ordinarily sensory potentials could be superimposed upon spontaneous waves, but that occasionally block occurred. Similarly, Dempsey and Morison (1942b) showed unequivocally that sensory potentials could occur with no reduction in size on any phase of the potentials of the spontaneous burst, and that these latter effects were intimately related to activity in medial rather than lateral thalamic areas.

If, as in this and preceding papers, "spontaneous" activity be regarded as a sum of several components rather than as a unitary phenomenon, there is a considerable resolution of the difficulties outlined above. For example, no contradiction exists between Bishop's statement that spontaneous potentials block an induced response and Dempsey and Morison's finding that evoked potentials may occur at any phase of spontaneous waves if it is assumed that in the former instance the spontaneous activity is of the projection type (p. 290) and in the latter the burst type. The observation by Bartley (1936) that optic stimulation induced a repetitive response which interacted with further stimuli supports the inference that projection activity is easily evoked in the visual system. Likewise, it appears probable that Lewy and Gammon were dealing with projection type

activity since it was influenced so markedly by section of the sensory tracts, while Bremer and Dusser de Barenne and McCulloch found that burst activity remained after intercollicular section. The interlinking of the cortex and ventrolateral thalamus observed by Dusser de Barenne and McCulloch after local strychninization, on the other hand, was clearly due to the projection circuit, since it was distributed to the lateral rather than medial thalamic areas and since it has been found that such cortical strychninization results in the firing of the strychnine spike whenever a sensory potential is induced in the strychninized area, while the potentials of the spontaneous burst do not trip the strychnine spike with such facility (Morison and Dempsey, unpublished data). Similarly, it appears probable that Dubner and Gerard were dealing with projection type activity in the lateral geniculate, since they observed a decline in magnitude on confinement to the dark and a re-establishment of activity on visual stimulation.

There are certain implications in the data presented above which bear upon the interpretation of cortical responses. Lorente de Nó (1939) has adduced evidence that an impulse traveling toward an "active" electrode induces in it a potential whose sign is positive when referred to a remote "indifferent" electrode. This interpretation has been followed by Curtis (1940) and Marshall, Woolsey and Bard (1941), although the latter authors observed that lemniscal stimulation produced in the cortex spikes of axonal dimensions followed by a positive wave. The evidence presented in figure 3 indicates that as opportunity for temporal dispersion is decreased, the surface positive component of the primary becomes resolved into two positive elements. The second of these elements declines faster with frequency than does the first. Likewise, the concomitant presence of an augmenting response causes a faster decline in element 2 than can be accounted for by fatigue of the afferent fiber (cf. fig. 2, Morison and Dempsey, 1943). These are phenomena which are difficult to explain on the assumption that both positive elements are the immediate electrical signs of corticopetal impulses. Rather, such discontinuities ordinarily attend transmission across a synapse, and it is therefore suggested that at least one cortical element must be activated during, and must contribute to, the surface positive wave which results from sensory stimulation.

SUMMARY

Stimulation of sensory elements at any level in the medial lemniscus-internal capsule relay system is followed by responses of three types in the sensory cortex and ventrolateral thalamic nucleus: 1, a "primary" response consisting of two positive and one negative deflections (figs. 1A and 3C and D) which follow rapid frequencies of stimulation; 2, an "augmenting sensory response" consisting of a positive and negative component which, although absent on the first stimulus, grows dramatically in size on successive stimulations (figs. 1B, 2A, 3A and 4); 3, a "repetitive sensory response" consisting of a train of potentials at a frequency of 8–12 per sec. which follows the primary response to a single stimulus and which fatigues quickly on repeated evocation (fig. 7A).

The primary and the augmenting responses have been separated from one another on the basis of latency (figs. 1 and 3), anatomical localization (fig. 2), selective abolition by lesions and differences in physiological behavior (p. 288).

Similarly, the separability of the primary and the repetitive responses has been demonstrated by the failure of one to block the other (fig. 6). On the other hand, the simultaneous presence of the repetitive response blocks the appearance of augmentation (figs. 7B and C).

The repetitive response, although superficially similar to the spontaneous burst potentials of the cortex, may be separated from them since the burst potentials occur in different areas in the thalamus (fig. 5) and since the presence of burst potentials does not prevent the evocation of repetitive responses (fig. 8). The validity of this finding is further indicated by the fact that the repetitive response is not affected by simultaneously induced recruiting potentials or by high frequency stimulation of intralaminar thalamic areas (fig. 12), although these procedures have been shown previously to interfere with the burst potentials. Similarly, the augmented response is not blocked by the burst potentials (fig. 9).

Evidence is presented that a type of spontaneous effect, the "projection activity," results from activity in the thalamic relay nucleus and its cortical projection. The projection activity differs from the spontaneous cortical bursts in regularity, susceptibility to anesthesia, thalamic localization (fig. 11) and dependence upon afferent impulses (fig. 10). Its interaction with the repetitive sensory response is complex (fig. 11B). It is abolished by rapid augmenting stimuli (p. 292), whereas augmenting responses are superimposable upon the burst potentials (fig. 9).

It is suggested that the recognition of different types of "spontaneous" activity facilitates study of the interaction of cortical potentials with induced effects. The bearing of these results upon various contradictory statements in the literature is discussed.

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MECHANISM OF THALAMOCORTICAL AUGMENTATION AND REPETITION

R. S. MORISON AND E. W. DEMPSEY

From the Department of Anatomy, Harvard Medical School, Boston, Mass.

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In another paper two types of cortical activity elicited by stimulation of the sensory path have been described. Evidence was presented that these phenomena, the so-called augmented and repetitive sensory responses, share a single neuronal mechanism, but may, on the other hand, be segregated both from the simple "primary" sensory response and the generalized 8 to 12 per second "spontaneous" bursts (Dempsey and Morison, 1943). The present study was directed toward a more thorough investigation of the properties of the system responsible for the augmented and repeating effects.

METHODS. The methods employed were entirely similar to those employed in the preceding papers and need not be repeated.

RESULTS. *Site of repetition and augmentation.* Since repetition is not a feature of sensory responses recorded from the lemniscus (Dempsey and Morison, 1943), attention was directed to the thalamus and cortex. Adrian (1941) has demonstrated that the thalamus deprived of its cortex still exhibits repetitive responses. In the present investigation records taken from the thalamus before and after cutting of the internal capsule showed that the repetitive response of the thalamus, though still present after the procedure, was always depressed. In order to facilitate cutting of the capsule in this and other types of experiments, the ventricle was carefully unroofed before any observations were made, so that the cutting of the capsule could be carried out with a minimum of additional operative trauma. Since depression of the thalamic response might still be attributable to some nonspecific effects of the operation, however, the influence of the cortex on the thalamic response was tested in another way. Cortical repetition was enhanced by local application of acetylcholine (Chatfield and Dempsey, 1942). The thalamic repetitive response was enhanced *pari passu* (fig. 1).

The question still remained as to whether the cortex alone may exhibit repetitive or augmented sensory responses. In order to solve this question, stimulating electrodes were introduced into the capsule and the cortical responses recorded. Division of the capsular fibers caudal to the electrodes abolished both the repetitive and augmented (fig. 2) responses. Even though acetylcholine was applied to the cortex in the hope of compensating for the loss of an asynchronous thalamic bombardment (cf. its facilitating effect in intact animals above), the abolished repetitive response did not return.

The primary response induced by capsular stimulation invariably was smaller and its second and third component declined more rapidly with frequency before section of the thalamic radiations. As the augmenting response developed (fig. 2A), a decline in the primary response occurred. Figure 2B shows the larger

size and greater durability of the primary effect after section of the thalamic radiations.

At this point it may be recognized that capsular stimulation could presumably involve both dromic and antidromic activation of either corticopetal or corticofugal elements. Difficulties arising from antidromic stimulation have been so slight as to justify their complete neglect. Phenomena of short latency (0–1 msec.) and duration (1–2 msec.) did indeed occur both in the cortex and thalamus as a result of capsular stimulation (cf. Dempsey and Morison, 1943), but they had little effect upon the later augmented and repetitive responses. These early effects in the cortex were presumably due to direct activation of the thalamocortical fibers along with an undetermined amount of antidromic firing of corticopetal elements. At all events, it seems unlikely that a very large propor-

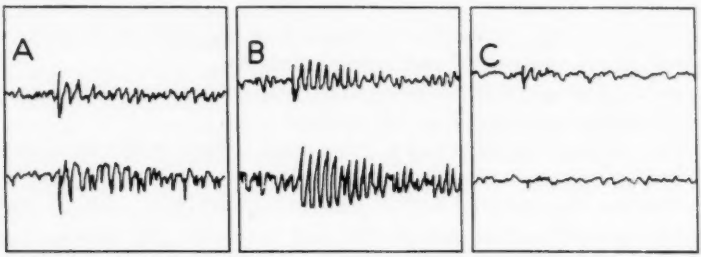

Fig. 1. Reinforcement of repetitive response by local application of prostigmine and acetylcholine to the cortex. Upper record, *n. ventralis lateralis pars externa;* lower record, radial cortical projection, surface bipolar recording. Paper speed, 10 mm. per second.

A. Immediate and repetitive effects in thalamus and cortex after single shock applied to the radial nerve.

B. After local application of prostigmine and acetylcholine to the cortex. The repetitive effect is enhanced in both cortex and thalamus.

C. After decortication by section of the internal capsular fibers. The thalamic repetitive effect, although still present, is greatly depressed.

tion of fibers carrying the augmented response to the cortex are activated directly by the electrodes in the capsule for the augmented response was typically very much larger than any early response to capsular stimulation. Indeed, from certain positions in the capsule virtually no cortical response other than the augmented one was detectable (cf. Dempsey and Morison, 1943).

Further evidence against attributing a significant rôle to direct activation of thalamocortical neurons is found in the observation that neither augmented nor repetitive responses could be produced in the cortex deprived of its thalamus. On the other hand, augmented and repetitive effects in the thalamus were easily elicited by capsular stimulation whether or not the cortex were present. The latency of the augmented response in the thalamus (2–3 msec.) was such that it preceded by an appropriate interval the corresponding cortical effect (3–5 msec.), an observation which lends cogency to the view that capsular stimulation exerts its effects on the cortex via the thalamus and not *vice versa* (fig. 3). Other

evidence which serves to establish the view even more satisfactorily may be found in the subsequent experiments.[1]

Rhythmic responses to high frequency stimulation. Relatively rapid frequencies applied either to the capsule or an afferent nerve resulted in a marked reduction of both the cortical and thalamic responses, a phenomenon due apparently to the

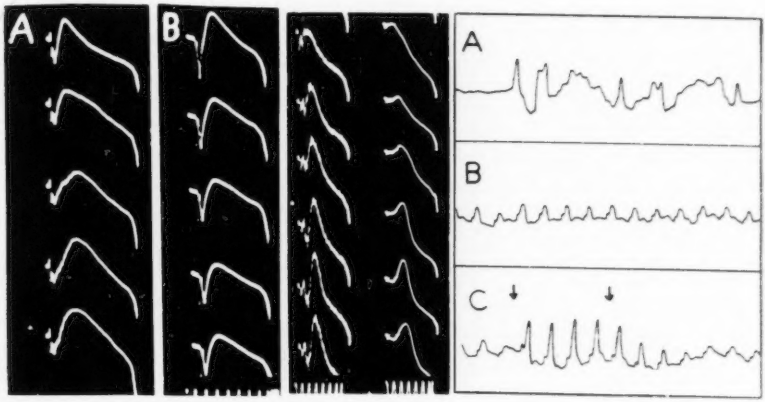

Fig. 2 Fig. 3 Fig. 4

Fig. 2. Abolition of augmenting response after removal of the thalamus.

A. Stimulus to the internal capsule. Primary and augmenting effects are present.

B. Same after section of the internal capsule posterior to the stimulating electrodes. The augmenting effect does not appear. The primary response is larger and does not decline so rapidly as in record A. Time signal, 250 cps.

Fig. 3. Simultaneous augmentation in thalamus and cortex during repetitive stimulation of the internal capsule. Left record, *n. ventralis lateralis pars externa*. Right record, radial cortical projection, surface to deep electrodes. Time signals, 100 cps. Note that the latency is shorter in the thalamic response.

Fig. 4. Interaction between capsular and afferent nerve stimulation. Radial cortical projection, surface bipolar electrodes. Paper speed, 40 mm. per second.

A. Effect of 120 per sec. stimulation of radial nerve. Note irregular repetitive discharges.

B. Equilibrated response to 12 per sec. stimulation of an augmentor point in the internal capsule.

C. One hundred twenty per sec. stimulation of radial nerve between arrows. Note increased responses to the capsular stimuli, and that this effect outlasts the period of nerve stimulation.

combined effects of fractionation and "fatigue." Superimposed upon the equilibrated series in many experiments a series of rhythmic waves of slow (8–12/sec.) frequency appeared both in the thalamus and cortex (figs. 4A and 5).

[1] The analysis here and throughout this study has assumed that the growth of the augmented response is produced by increasing the number of active elements. Owing to the exigencies of space, the somewhat elaborate discussion necessary to justify this procedure is omitted.

Again the effects of both capsular, lemniscal and afferent nerve stimulation were similar, except that the former were easier to obtain and more marked in extent. The rhythmic waves occurred in the thalamus deprived of its cortex, but not in the cortex without the thalamus.

Interaction of afferent and capsular stimulation. It has been shown elsewhere that the simple sensory primary response elicited from afferent nerve stimulation is "blocked" by prior activation of the appropriate portion of *n. ventralis lateralis pars externa* or of the internal capsule (Dempsey and Morison, 1942). Similar experiments involving the augmented response revealed much more complex interactions. Complete block has never been unequivocally encountered, but various degrees of mutual facilitation or occlusion have been the rule. Facilitation was strikingly illustrated in some experiments by setting up a cortical response by capsular stimulation at a frequency high enough to result in marked equilibration. An added stimulation of an afferent nerve at rates so high that

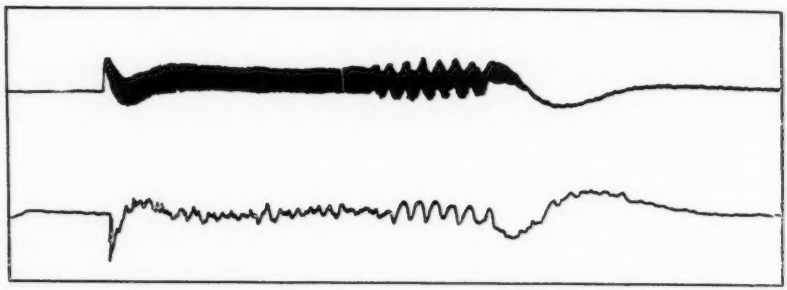

Fig. 5. Effect of high frequency stimulation of the internal capsule. Upper record, *n. ventralis lateralis pars externa*. Lower record, radial cortical projection, surface to deep recording. Paper speed, 15 mm. per sec. Stimulus frequency, 250 per sec. Note the development of oscillations in the records.

discrete responses to the individual volleys were undetectable yielded marked facilitation of the cortical response at the capsular frequency (fig. 4).

At slower frequencies the effects depended upon several factors including especially the time relations or phase angle between the two types of volleys. Less than lineal or greater than lineal addition of the effects were encountered in different conditions suggesting that the pool of thalamocortical neurons could be shared by the corticofugal and lemniscus fibers. Under conditions of marked equilibration or slight augmentation a large subliminal fringe was available to the other stimulus, and facilitation would result. With highly augmented or slightly equilibrated responses "occlusion" was revealed (i.e., less than lineal addition).

Time course of augmentation. One of the most interesting features of the augmentation effect was its time course. Figure 6 shows the effect of a second stimulus at varying intervals after the first. It is clear that augmentation was minimal shortly after the first stimulus and rose, first rapidly then more slowly,

to reach a maximum at 100 to 120 msec. immediately before the appearance of the first repetitive wave. All augmentation was abolished during the repetitive wave, but reappeared immediately afterwards to rise to another, frequently higher, maximum before the next repetitive wave and so on (fig. 7). The presence of the repetitive response made it difficult to determine the total duration of the augmentation produced by the first shock because the repetitive waves might have contributed to or subtracted from it.

Fig. 6. Timing of the repetitive response by the second of two stimuli. Radial cortical projection, surface to deep electrodes. Paper speed, 20 mm. per second.

A. Immediate and repetitive effects of a single shock applied to the internal capsule.

B. Response to two shocks separated by an interval of 100 msec.

C. Sixty-seven msec.; D. 50 msec.; E. 33 msec.; F. 25 msec.; G. 20 msec.

The first repetitive potential occurs at a constant time after the second stimulus, regardless of its phase relation to the first.

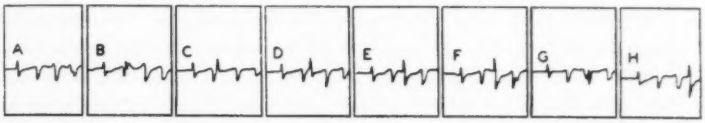

Fig. 7. Growth of augmentation during the interval between repetitive potentials. Radial cortical projection, surface bipolar electrodes. Paper speed, 20 mm. per second.

A. Immediate and repetitive responses to a single shock applied to the ventrolateral thalamic nucleus.

B. Block of augmentation when a second shock falls during the first repetitive potential.

C, D, E and F. Increase in augmentation as second shock falls later and later after the preceding repetitive potential.

G. Block of augmentation when second shock falls upon the second repetitive potential. Compare with B.

H. Augmentation when second shock falls after the second repetitive response. Compare with F.

Note that the repetitive effects are retimed by the second shock. The interval between the second shock and the next repetitive potential is constant, regardless of the phase at which the stimulus falls.

A somewhat similar waxing and waning of the responses of the optic cortex to the second of two stimuli has been discussed by Bartley (1936, fig. 3). In spite of the differences in cortical area, species, anesthesia and stimulating methods employed, the similarities between the two results suggest that a similar mechanism may be fundamental to both situations. It may be suggested that the second or slow response of Bartley and Bishop (1933) is analogous to the augmenting response identified here. As Bartley (1941) points out, the slow response is more intimately connected than is the earlier diphasic wave with spontaneous cortical activity, a point consistent with the augmenting effect of

Dempsey and Morison (1943, p. 291). On the other hand, it seems regularly to occur in the optic area in response to a single shock, whereas in the somatic sensory area it was more typically the result of repeated stimulation. This and other differences have led to rather different interpretations in the two cases, but it seems probable that as more information accumulates a single, general explanation may be possible.

In preparations without a repetitive response or in those in which it had been fatigued, augmentation as tested by two shocks usually recorded a maximum at 100 to 300 msec. and then slowly declined after the next second or two to normal, although in this condition the maximal possible effect was somewhat less than in the unfatigued preparation. Other facts which suggest that the early rising phase of augmentation is relatively uninfluenced by intercurrent discharges were brought out by the use of repetitive stimulation. At frequencies higher than that of the repetitive rhythm consecutive augmentation of the first several shocks was usually seen. Over a range of from 8 to 20 per second the curve relating height of the individual responses to time was relatively constant (cf. fig. 8) at least in its early phases. In other words, neither the slope of the rising phase nor the maximum height of augmentation was increased by increasing the number of stimuli per unit time At optimal frequencies the total duration was, however, longer than that produced by a single shock. As frequency increased, however, the curves of augmentation began to decline earlier, suggesting "fatigue" and the appearance of progressive fractionation. At frequencies above about 30 per second complexities appeared in the early part of the curve, suggesting that refractoriness and fractionation were the dominant factors.

Timing of the repetitive responses. Perhaps the most interesting feature of the repetitive response is the regularity of its timing. In the course of a single experiment the frequency of the beats remained surprisingly constant even when various procedures had greatly altered the height of each individual beat or the duration of the train.

The application of acetylcholine to the cortex greatly enhanced the responses both in individual height and in total duration not only in the cortex but also in the thalamus. The frequency of the discharge, however, was essentially unchanged. Conversely, repeated production of the repetitive response at intervals of about 3 to 5 sec. greatly reduced the duration of the discharge and the height of the constituent waves without influencing their frequency. Indeed as each individual discharge petered out, the waves declined in size but changes in the interval between them were insignificant. Furthermore, although the repetitive response was always greater when produced by stimulation of the internal capsule than as a result of afferent nerve activation, the frequency in both cases was similar.

The following summary statement appears to be justified. The mechanism responsible for the repetitive response is timed by some subsidiary mechanism relatively inaccessible to the excitatory state upon which duration of after discharge or the height of the individual waves depends.

Another extraordinary feature of the mechanism was revealed when two single shocks were given at varying intervals. The experiment illustrated in figure 6 in which the stimulating electrodes were in *n. ventralis lateralis pars externa* was typical; similar results have been obtained by the stimulation of afferent nerve or capsule. The first stimulus set up the repetitive train. No matter when the second shock was given and irrespective of how small the response elicited, the interval between it and the next succeeding repetitive wave was constant, i.e., the timing of the remainder of the train was set by the second stimulus. The total duration of the train as measured from the first stimulus, however, was but slightly if at all increased by the addition of the second shock. In other words, a second shock could alter the timing of the series but did not contribute significantly to the duration of the excitatory state.

As the stimulus interval was decreased below 20 msec. the second shock became less efficient at taking over the timing, and irregularities appeared in the repetitive waves suggesting that the pool of thalamic neurons was split between the two stimuli, but beyond this limit the rule outlined above was strictly followed. Similar results were obtained if three or even four shocks were used; the last one always set the remainder of the rhythm. In the multiple shock experiment the train was usually somewhat shortened or even completely abolished.

An exception to the statement made above that a second shock did not contribute to the excitatory state responsible for repetition should be mentioned. A weak stimulus to the capsule would sometimes be followed by a repetitive response smaller and shorter than "maximal." Such responses were increased by the addition of a second shock at some reasonable later interval. The gradations between submaximal and maximal responses were not smooth, but tended to occur in steps. This fact which suggests that the elements controlling repetition are relatively few in number was reinforced by other observations. Many of the repetitive discharges tended to show constant irregularities in the constituent waves which suggested that they were made up of two or more units firing slightly out of phase. As the discharge disappeared, the height of the original waves tended to decline by steps and not by a continuous decline (cf. Dempsey and Morison, 1943, fig. 7A). The splitting of the pool between two stimuli arriving a short time apart mentioned in the preceding paragraph also reinforces the suggestion that the repetitive mechanism is made up of relatively few "all-or-none" units.

Discussion. The experiments described in this and accompanying paper suggest that a system in addition to the simple lemniscus to internal capsule relay is present in the ventrolateral thalamus. It would appear that extensive afferents to this thalamic system are derived from the cerebral cortex and the medial lemniscus. Moreover, these afferents apparently do not end upon the ultimate thalamocortical neurons, but exert their effects through interneurons. Finally, this system of thalamic afferents, interneurons and efferent cells has certain properties, typified by a slowly rising excitatory state and by prolonged rhythmic activity, which are pertinent to a discussion of the possible anatomical and physiological organizations underlying the various phenomena.

In order to summarize the data and point up its implications, it may be helpful to consider a diagram (fig. 9). Clearly in such a complex system data can only be cited very tentatively as favoring one or another of the contested views in regard to the intimate nature of synaptic transmission, facilitation, c. e. s., c. i. s., or after discharge.

As Lorente de Nó (1939) has shown, many of the properties attributed by others to c. e. s. and "detonator" activity may be reduced to temporal and

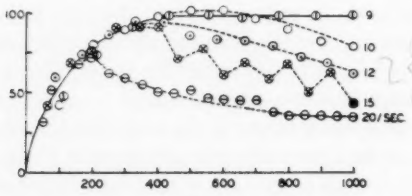

Fig. 8

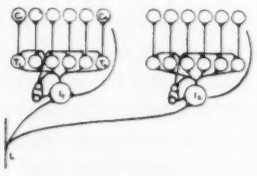

Fig. 9

Fig. 8. Time course of cortical augmentation at various frequencies. Ordinates, height of successive responses at indicated frequencies, expressed as per cent of maximal augmented response. Abscissae, milliseconds after first stimulus. The rate of increase in augmentation is independent of the frequency of stimulation, although the decline in height during equilibration is greater and occurs earlier as the frequency is increased.

Fig. 9. Schematic representation of the system responsible for augmented and repetitive effects.

A homologous group of interneurons, I_1, I_2 . . . I_n, interposed between capsular and lemniscal afferents and thalamocortical efferents, is postulated to fire on any afferent stimulus. These interneurons distribute to thalamocortical efferents T_1 . . . T_n, each element of which requires successively greater degrees of facilitation for its discharge. Since the degree of augmentation is a function of time rather than frequency of stimulation, the excitatory state is represented as a self-re-exciting chain which is started by the discharge of I and which facilitates transmission at T_n. Any afferent stimulus therefore fires I and the portion of T_n previously facilitated by the closed chain.

Since the system fires repetitively after a single stimulus, the closed chain is regarded as symbolizing the summatable excitatory state involved in rhythmic firing of I (see text). The discharge of I, so induced, fires all of T_n because its excitatory state induced by previous activity of the closed chain is maximal at the period of the repetitive effect. This group discharge of T_n abolishes its excitatory state thus temporarily abolishing augmentation to an interpolated shock. Furthermore, the entire system is retimed by the abolition of facilitation at I following its discharge (see text).

The origin of return fibers from the cortical cells C_n is unspecified, since it is probably several neurons removed from the element on which the thalamocortical fibers terminate.

spatial patterns of activity in interneurons. Although the phenomena presented here can apparently be explained in these terms, such a diagram becomes very complex, and, as Lorente de Nó has pointed out, loses therefore much of its didactic value. A rigidly complete analysis in those terms has therefore not been attempted.

The key to the present system lies in the fact that the timing mechanism of the rhythm of the repetitive response bears a highly specialized relationship to the afferent fibers (either capsular or lemniscal) on the one hand, and to the

efferent or thalamocortical fibers on the other. The principal point of interest is that the entire system may be retimed by stimuli which may activate relatively few of the thalamocortical fibers (fig. 6). It seems desirable, therefore, to postulate at least one group of homologous interneurons I_n between afferents and efferents, which may be thought of as completely accessible to successive volleys arriving at intervals so short as to be within the unresponsive period of the thalamocortical pool T_n. This group may be taken as symbolic of a much more complex interneuronal system, primarily concerned with control of the rhythm.

The fact that the rhythmic waves of the repetitive response could be resolved in favorable instances into a relatively small group of units which tended to appear or disappear in a relatively "all-or-none" manner has suggested the representation of the interneuronal system as composed of a few large cells receiving many corticothalamic and lemniscus afferents and relaying to a large number of thalamocortical efferents. The smoothly graded nature of the augmented response may then be attributed largely to recruitment in the large pool of the latter type, although facilitation at both I_n and T_n would of course be important with weak capsular stimuli or in the case of activation of afferent nerves (cf. p. 303). For conveniently analyzing recruitment at T_n, stimuli maximal for I_n are therefore desirable, and the data detailed in figures 6 and 8 have been obtained with intensities such that temporal effects at I_n may be neglected. Started by a single volley, augmentation slowly rose for 100 to 300 msec. and then slowly disappeared. The rising phase and total amount of augmentation have further been shown to be relatively uninfluenced by additional volleys, although the duration of the effect may be prolonged by repetitive stimulation (fig. 8).

The excitatory state responsible for augmentation has, therefore, been represented as a self-re-exciting chain which is started by a single shock and continues to run for 2 to 3 seconds with a rate uninfluenced by successive volleys within that interval. Activity in this system may be thought of as progressively lowering the threshold of T_n so that more and more of them become available to the second volley arriving over I_n. A strict interpretation of the concept of Lorente de Nó would apparently rule out such an accumulation and a more complicated system of delays would be necessary. On the other hand, the shape of the time curve and the lack of evidence for actual firing of T_n by the facilitating process alone suggest the c. e. s. postulated by Eccles (1936).

It is now possible to consider the characteristics of pool I_n which is made responsible for the rhythmic properties of the system. In addition to relaying impulses to pool T_n as outlined above, pool I_n is postulated as firing rhythmically under proper conditions. At present the whole problem of rhythmicity can only be discussed in vague terms, but the phenomena considered here are most readily understood if one assumes that the rhythmic firing of I_n is due to the interaction of an extrinsic excitatory effect represented by a collateral from the reverberating side chain already postulated to account for facilitation at T_n, and a non-summatable recurring refractoriness or subnormality of constant

duration. The arrangements necessary for rhythmic activity are clearly more specialized and labile, however, than are those of the simple relay activity. In general more impulses must reach I_n to elicit the repetitive response and it is distinctly more affected by fatigue. No matter what the mechanism of rhythmicity, the accurate retiming of the system by a second shock raises an interesting point (figs. 6 and 7, p. 301). All the interneurons controlling T_n are affected by the interpolated shock whether or not T_n are fired thereby since no wave appears at the usual interval after the first stimulus. On the other hand, the entire pool fires repetitively at the usual interval as measured from the interpolated stimulus. The constancy of the interval between interpolated discharge and first repetitive wave differentiates the phenomenon from the retiming of rhythmic motor neuron discharge described by Eccles and Hoff (1931). In their instance the interval succeeding the interpolated discharge varied inversely with the length of the curtailed cycle, a situation which appears easily explicable on the basis of summation of subnormality or an interaction of N and P waves (Eccles, 1936). The present case, though superficially simpler, is actually more difficult to explain, and necessitates the non-summatability of the subnormal state postulated above, which just cancels the accumulated facilitation.

The fact that repetitive responses are more difficult to elicit by afferent nerve or lemniscal stimulation than from the capsule has led to the supposition that the lemniscus fibers relaying impulses from a particular afferent nerve are sparsely represented on the I_n cells. On the other hand, when properly facilitated (i.e., by application of acetylcholine and prostigmine to the cortex) a very large part of I_n becomes available. The inference is that any portion of the lemniscus though poorly represented on any particular part of I_n is distributed widely through the pool. Cross linkages between the interneurons I_n could also contribute to the spread of effects and are rendered quite probable by the synchronization of the waves in the repetitive response and the presumably similar rhythmic waves produced by high frequency stimulation. A mechanism for avalanche conduction and the more elaborate interpretations discussed by Marshall and Talbot (1941) is provided by either sort of connection. Clearly also an opportunity for the various forms of interaction between lemniscal and capsular stimulation outlined on page 300 is offered by the overlap of these two types of afferents on the I_n cells.

It is interesting to note that although interactions between capsular and afferent nerve stimulation reveal various states of facilitation as well as occlusion of the augmented response, the true primary (elements 1, 2, 3 of Dempsey and Morison, 1943) exhibits occlusion only (Dempsey and Morison, 1942). This and other evidence such as the independence of the primary response from spontaneous activity in any recognized corticothalamic circuits suggest that corticothalamic fibers are poorly represented on the neurons responsible for the relaying of the simple primary response.

Presumably the repetitive waves recorded in response to high frequency stimulation of either capsule or afferent nerve (p. 299) is due to a piling up of excitation at these interneurons. At these high rates responses of the I_n cells to the indi-

vidual volleys seem to be prevented probably by the postulated subnormality which allows only the much slower rhythmic waves to appear in response to an accumulated excitatory state.

In conclusion it may be worth while to turn for a moment to the problem of corticothalamic interaction. From the evidence presented (p. 297) it is clear that the principal mechanism underlying augmentation and repetitiveness lies in the thalamus. The fact that the rhythm of the repetitive response of the thalamus is unaltered by decortication (p. 298) emphasizes that the timing is localized in the thalamus and is not a property of the circuit as a whole. On the other hand, the cortex contributes importantly to the intensity of the response and to its duration, presumably by facilitation fed back to I_n and perhaps to T_n. Thus, though the circuit may not be strictly "reverberating," it may properly be referred to as mutually reinforcing. The relation of this system to an element of the spontaneous electrocorticogram and the fundamental experiments of Dusser de Barenne and McCulloch is discussed elsewhere (Dempsey and Morison, 1943).

CONCLUSIONS

Investigation of the mechanism underlying the production of repetitive and augmenting sensory responses has revealed the following:

Decortication reduced but did not abolish the repetitive response recorded from the thalamus. Enhancement of the cortical response by local application of drugs increased the thalamic effect (fig. 1). Removal of the thalamus abolished the repetitive and augmented responses evoked by capsular stimulation. These and other experiments (cf. figs. 2, 3, 4, 5 and p. 300) show that extensive thalamic afferents are derived from the internal capsule as well as the medial lemniscus. It is concluded that in this system there is a mutual reinforcement of cortical and thalamic effects, but that the thalamus alone is necessary for the occurrence of repetitiveness and augmentation.

Augmentation induced by a conditioning shock, and revealed by a second test shock, extends over several hundred milliseconds (p. 301; figs. 6, 7 and 8). Furthermore, once the process is started, it proceeds at a rate which is not influenced by subsequent stimulation (fig. 8).

Repetitive sensory potentials induced by the conditioning shock destroy augmentation. Following the repetitive effect, the process of augmentation again builds up to a maximum just before the next repetitive response (fig. 7).

The timing of the repetitive rhythm bears a highly specialized relationship to the afferent stimuli. The rhythm is timed by the last stimulus, whether it falls during the repetitive train (fig. 7) or ahead of the first repetitive potential (fig. 6). Likewise, the rhythm is set by the last shock both when the number of thalamocortical elements fired is small (fig. 6 E, F and G) and large (fig. 6 B, C and D).

The data presented above imply certain properties of the thalamocortical system which are summarized (p. 303) and discussed with reference to a circuit diagram (fig. 9) whose elements are to be regarded as symbolizing such physiological phenomena as the excitatory state, detonator action and repetitiveness.

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CORRELATION OF VASCULAR CHANGES WITH CHANGES IN MOTOR ACTIVITY AND SECRETION IN THE STOMACH OF MAN

STEWART WOLF[1] AND HAROLD G. WOLFF

From the New York Hospital and Departments of Medicine and Psychiatry of the Cornell University Medical College, New York, N. Y.

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The subject of our investigation was a man with a permanent gastric fistula 3.5 cm. in diameter, larger than that of Alexis St. Martin (1) or any other which has been studied in the past. His defect was the result of an operation done in 1895 and made necessary by a benign stricture of the esophagus. Despite the large communication to the outside world the appearance of the gastric mucosa both by direct inspection and gastroscopically was found to be normal (2). Secretory and motor functions of the stomach were average (2). Protruding through the defect and reflected on the abdominal wall around it is a collar of gastric mucosa. The width of this collar is made to vary with change in intra-abdominal pressure.

METHOD. Our subject came to the laboratory in the morning in a fasting state, and reclined for one-half hour on a cot before observations were begun. An effort was made to keep the subject's surroundings as neutral as possible and to keep him lightly diverted.

Circulatory changes. Changes in vascularity were readily recognized by variations in the color of the gastric mucous membrane. It was seen to undergo a very wide range of color changes under various circumstances, from a pale yellowish pink to a deep cardinal red. Changes of the same order and in the same direction occurred in the part of the mucosa within the cavity of the stomach and in that which lay exposed on the abdominal wall. Since it was simpler to obtain ideal lighting conditions on the outside, and since there a color scale could be brought up close to the mucosa for comparison, the recorded vascular changes are those observed in the collar of exposed mucous membrane under a cool, "soft white" fluorescent light suitably placed to provide constant lighting conditions. The standard colors used for comparison were quantitated by the method of Munsell. For convenience, however, the colors were given values from 10 to 100. The lower numbers corresponded to pale colors and the higher ones to deeper shades of red. It has been shown by a modification of the thermal gradient technic, described elsewhere (3) that these color changes do in reality reflect changes in blood flow.

It is important to note that accelerated blood flow in the mucosa was not merely associated with blushing of its surface. The membrane itself became wet, swollen, and turgid, and the rugae were slightly fuller and smoother. These evidences of vascular engorgement were especially obvious in the collar

[1] National Research Council Fellow in the Medical Sciences.

of mucosa exposed on the abdominal wall. During marked hyperemia, it often doubled in thickness from 5 mm. to 10 mm. and its radial folds decreased in number from 12 or 13 to 5 or 7. The tissue itself under such circumstances felt succulent and boggy.

Recording of gastric contractions. Records of contractions were obtained by the familiar technic of recording pressure changes in a balloon introduced into the stomach. It must be emphasized that this type of recording device measures only those contractions which alter the intragastric pressure. It has been suggested that certain peristaltic waves may course along the stomach without causing a change in intragastric pressure (4).

Collection of gastric juice. Continuous aspiration of the stomach contents was carried out through a small rubber tube accompanying the balloon. Collections made thus, when the walls of the stomach were held apart by the presence of the balloon, effected satisfactory emptying of the stomach. In the absence of the inflated balloon, however, the gastric juice collected in isolated folds of the collapsed stomach and was thus not thoroughly accessible to aspiration through a tube.

The completeness of the collection when the balloon was in place was confirmed by removing the balloon after collection of secretion through the tube, and rolling the patient over on his left side approximately 120°. No additional fluid poured out.

Analysis of gastric juice. The gross appearance of the specimens was noted. The degree, if any, of discoloration with bile was recorded. The relative concentration of mucus was estimated by noting the comparative viscosity of the fluid and the presence of shreds. Peptic activity was determined by the method of Mett (5). The concentration of acid was estimated by titration of the specimens against 0.1 N sodium hydroxide with Toepfer's solution and phenolphthalein as indicators of "free" and "total" acidity, respectively.

Estimation of parietal cell output. No method was available for the estimation of the output of hydrochloric acid by the parietal cells. Since acid is manufactured only by the parietal cells, however, it seemed clear that total hydrochloric acid was some function of volume of juice secreted within a specified time and the concentration of acid. Based on the relationship between hydrochloric acid and neutral chlorides in gastric juice established by Hollander (6), Gray (7) and others (8) (9), a formula was devised for the calculation of the approximate output of the parietal cells in terms of cubic centimeters of 0.166 N hydrochloric acid.

Reproduced in figure 1 is a modification of the curve established by Hollander for the relationship between hydrochloric acid and neutral chlorides in the gastric juice. Reference to the figure will show that the lower end of the line which relates the 2 constituents points near 166 millimoles of hydrochloric acid (total titratable acid of 166). A specimen of gastric juice containing this concentration of acid would consist of pure parietal secretion. At the other end of the curve, the constituents of the alkaline component are at a maximum, and there is no parietal secretion present. At a point half way along the ordi-

nate and abscissa, we are dealing with a solution containing a 50 per cent concentration of each of the constituents. Perpendiculars dropped from each of these points intersect at the midpoint of the curve. Therefore, at suitable intervals along the curve, perpendiculars may be dropped onto the abscissa to denote what per cent of solution of certain titratable "total acid" is actually made up of parietal cell secretion. Figures for total acid, of course, correspond numerically to millimoles of hydrochloric acid. Multiplying the percentage figure by the volume of gastric juice secreted within a given time yields the approximate quantity of parietal cell secretion elaborated during that period.

Sources of error in calculation for parietal cell output. The value thus obtained would, of course, not be entirely accurate since the curve illustrated was estab-

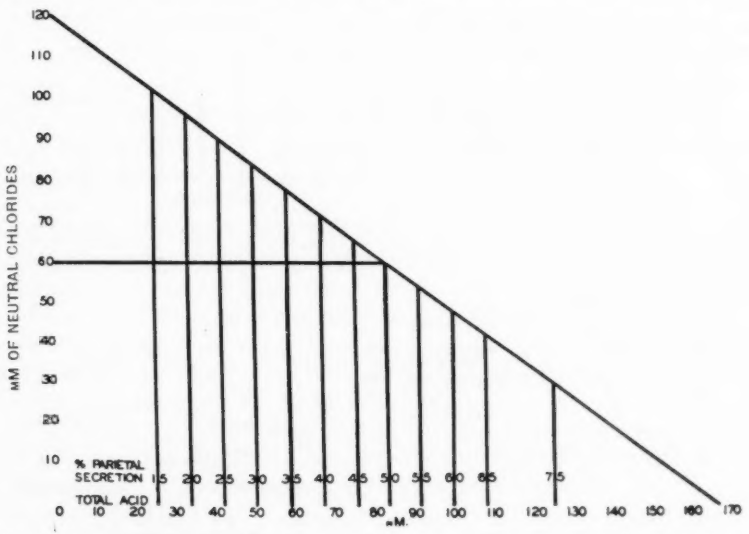

Fig. 1. Curve illustrating the relation between neutral chlorides and hydrochloric acid in the gastric secretion. (Modified from Hollander.)

lished on the basis of a statistical relationship, and individual specimens must be expected to vary slightly from the mean. Furthermore, the calculations were based on analyses of pure gastric juice in a blind stomach pouch. Any admixture of the juice with saliva or bile or any loss of juice through the pylorus would introduce a source of error. In this particular case, of course, the gastric juice could not be contaminated by saliva since the esophagus was completely occluded. Contamination of the juice with duodenal contents was easily recognized by the bile tint and such specimens were not included in calculations of parietal cell activity. Loss of secretion by emptying through the pylorus, however, was a persistent source of error. It always influenced the calculations in the same direction, however, and since emptying occurred only during periods of active motility (2), in the absence of vigorous gastric contractions the error became negligible.

CORRELATION OF FUNCTIONS. 1. *Basal state.* Carlson (10) has shown that during the resting "basal" state the stomach undergoes periodic phases of intense contractile activity alternating with phases of relative quiescence. This same periodicity of function we have noted in the activity of the acid secreting cells and in the vasomotor activity in the stomach.

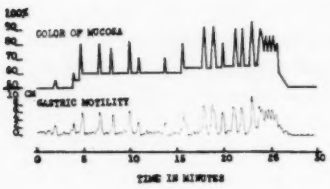

Fig. 2. Typical pattern of a phase of accelerated gastric motility with characteristic ending in a period of incomplete tetanus. Note changes in color of the mucosa associated with vigorous contractions.

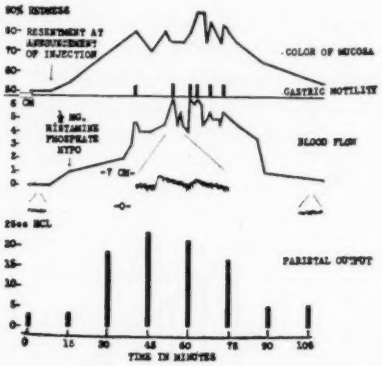

Fig. 3. Correlation of gastric functions including recorded blood flow following histamine injections.

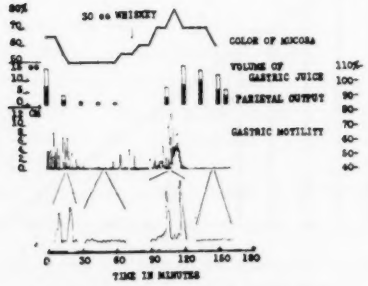

Fig. 4. Effect of alcohol on gastric function. Whiskey was administered following a period of spontaneous accelerated gastric function. Normally, a second such period would not follow for at least 2 hours.

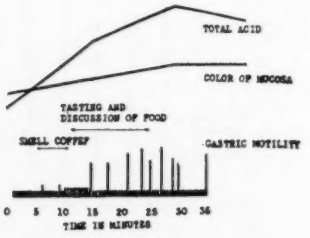

Fig. 5. Gastric function associated with stimulation of appetite.

Figs. 2-5

In the above figures the designation "Parietal output" indicates the volume of HCl at concentration 0.166 N.

The "basal" waves of low amplitude were not found to be accompanied by any detectable change in the color of the mucous membrane of the stomach. The more vigorous contractions, however, were associated with a simultaneous transitory blushing of the mucosa proportional in degree to the force of the contraction. Figure 2 shows the correlation of color changes in the mucosa with vigorous contractions of the stomach wall.

Like blood flow the rate of acid production in the stomach was accelerated

during phases of vigorous contractions and relatively slow during quiescent phases.

Comment. The occurrence of these spontaneous periodic phases of accelerated gastric function was carefully taken into account when vascular changes and changes in secretion and motility were attributed to the influence of stimuli applied experimentally. Usually such stimuli were applied shortly after the termination of one of these phases, when another would not be expected for an hour and a half. In addition, the experiments were repeated sufficiently often to establish the validity of the inferences.

Agents well known to cause an acceleration of acid secretion were tested for their effects on motor activity and vascularity in the stomach.

Effect of histamine on blood flow, acid secretion and motor activity. During a half-hour control period the gastric juice was collected and the color of the mucosa was observed. In addition a thermal gradientometer described elsewhere (3) was in place inside the stomach to measure changes in blood flow. Thus it was possible to correlate blood flow with color changes.

After the establishment of a base line, 0.0005 gram of histamine phosphate was injected hypodermically.

The results are charted in figure 3. Within 5 to 10 minutes of the injection the red color of the mucosa became deeper and there was a parallel increase in recorded blood flow. At the same time acid output increased in terms of parietal cell secretion and total titratable acid. It can readily be seen in the figure that the parietal cell output parallels the vascular changes.

Within 25 minutes after histamine injection strong contractions of the stomach wall occurred. With each of these there was a transitory blushing of the mucosa and an increase in recorded blood flow.

The maximum effect from histamine in terms of secretion, blood flow and motility occurred in about 45 minutes. After 90 minutes the effects had nearly subsided and the values had returned to normal by 2 hours.

Comment. These data illustrate: 1. That vascular changes observed as variations in color of the exposed gastric mucous membrane actually reflect changes in blood flow. 2. That histamine stimulated an increase in blood flow and motility as well as in acid production. 3. The importance of expressing acid output in terms of parietal cell secretion, since the "total acid" concentration failed to fall to control levels following a phase of accelerated secretion when acid output had subsided.

Effects of alcohol. After establishing a base line of vascularity, secretion and motor activity, 30 cc. of 90 proof whiskey (45 per cent alcohol by volume) was introduced into the stoma. As in the case of histamine an acceleration of all three functions occurred. The maximum effect was observed in 45 minutes, and in 90 minutes the values had returned to normal. A typical experiment is illustrated graphically in figure 4.

Comment. Other workers (10, 11) have shown that alcohol need not be introduced directly into the stomach in order to stimulate the parietal cells to accelerated activity. Given intravenously or rectally the drug causes a

sharply increased output of acid. Our observations indicate that even when ingested, the effects are not the result of local stimulation. Hyperemia of the mucosa and accelerated secretion did not occur until the drug had had time to become absorbed.

Effects of beef juice. Similar observations were recorded when a beef bouillon cube was introduced into the stoma. Within 15 minutes there occurred a flushing of the mucosa with enhanced secretory and motor activity which lasted more than an hour.

Preparation for eating. Another circumstance known to be associated with accelerated acid output is the feeling of good appetite which accompanies the sight or mere thought of delectable food. This was found to be also associated with hyperemia and hypermotility as illustrated in figure 5. The vascular engorgement, accelerated acid production and increased motor activity occurred promptly following the mention of appetizing food. Actual presentation and tasting of the food added little to this effect.

Hunger. Although, occasionally, hunger pains were reported by the subject at times in the absence of stomach contractions (2), as a rule they were found to be accompanied by vigorous contractions and a relatively red, actively secreting mucosa.

CORRELATION OF BLOOD FLOW, ACID PRODUCTION AND MOTOR ACTIVITY. *General statement.* While profound changes in blood flow, acid production and motor activity have been observed accompanying various emotional states and other circumstances, never in this subject were changes in vascularity and acid secretion dissociated. An increase in one was found under all circumstances to be associated with an increase in the other. Conversely, low acid output and pallor of the mucosa have been regularly associated. Vigorous motility has never been observed when the color of the mucosa was 50 or below. In the presence of red mucosa, vigorous contractions might or might not occur. No correlation was observed between the degree of hyperemia and the height or frequency of contractions, but accompanying each one was noted a transitory increase in redness of the mucosa, which subsided with the relaxation of the stomach wall.

DISCUSSION. Doubtless, enhanced secretory and motor activity in the stomach requires added work by the cells of the stomach wall. Davenport and Fisher (13) have shown that in secreting acid, work is done by the cells of the gastric mucosa to the extent of 800 calories per liter of secretion. Others (14, 15, 16, 17) have found in animals and humans low values for gastric acidity during states of anoxemia induced by high altitude, low oxygen tension chambers severe hemorrhage, and even polycythemia. Correction of the anoxic state resulted in a return of acidity values to normal.

All of this suggests that hyperfunction of the stomach would be accompanied by hyperemia, and indeed it is a general biological experience that heightened cellular metabolism requires increased blood flow.

A few investigators (18, 19) have recorded combined measurements of blood flow and gastric secretion in animals. Lim, Necheles and Ni (18) studied gastric blood flow in the excised dog's stomach, perfusing it with another dog's

blood. They demonstrated acid secretion following histamine injections. The blood flow, as measured by cannulating the veins, failed to parallel the rate of secretion.

Dodds and his co-workers (19) made similar studies on dogs after surgical intervention. Instead of resecting the stomach, they left it in place and resected the entire small and large intestine. Following this, appropriate vessels to the stomach were cannulated, and the blood flow was measured by collecting the venous blood in a graduated container for a certain length of time. The blood was then transfused back into the animal. Their findings differed widely from those of Lim et al. An increase in blood flow was noted with an increase of volume of gastric juice secreted in response to histamine injection. They were able to inhibit the histamine effect with regard to volume of gastric secretion and blood flow by giving injections of pitressin or barium chloride, or by causing a vasoconstriction in the stomach by irrigating it with cold water. The titratable acidity, however, remained high.

In the interpretation of all of these animal experiments, one must reckon with the trauma of surgical operation, the effects of anesthesia, and the pathological changes secondary to the operative procedure.

A few observations have been made on circulatory changes in the gastrointestinal tract by direct inspection of the serosal and mucosal coats. Kuntz and Hazelwood (20) found that a reflex vasoconstriction in the serosal coat of the intestine of the cat was induced by packing ice on the abdominal wall. Warming the abdomen was found to cause a vasodilatation in the serosa.

Beaumont (1) recognized in Alexis St. Martin that the stomach lining was redder at some times than at others. "On the application of aliment," he wrote, "the action of the vessels is increased, the color brightened. . . ." Anger and fear, he noted, produced a "morbid" appearance of the mucosa, although it is not entirely clear from his notes whether under those conditions the stomach was redder or paler. Carlson (10) observed in his Mr. V. a deepening of the red color of the mucosa with each strong contraction of the stomach wall. Schindler (21) has described the appearance of the stomach mucosa of patients with peptic ulcers as appearing redder than "normal" through the gastroscope. This instrument has not been left in place long enough to follow changes in color for more than a few minutes at a time.

Drury, Florey and Florey (22) studied vasomotor changes in a patch of exteriorized colon in dogs. They found with alarm or fright a uniform blanching of the mucosa, due presumably to constriction in the large branches of the mesenteric arteries. Barcroft and Florey (23) using similar preparations found pallor marked during brief spurts of running, but less marked during prolonged exercise. They also found pallor in association with "anxiety" or "excitement."

Our studies support these findings—that profound vascular changes occur in the gastric mucosa and, in addition, that hyperemia occurs in association with accelerated acid secretion and motor activity, and pallor with a decrease in these functions.

SUMMARY. Simultaneous observations of motor activity, vascularity, and

secretion in the stomach have been recorded. During the resting state, the stomach was found to be relatively pale and contracting with rhythmic waves of low amplitude. Acid was secreted continuously in small amounts. From time to time there occurred spontaneous phases of hyperemia, hypermotility and hypersecretion.

The effects of histamine and other stimuli on the various gastric functions were recorded. A direct association between vascularity (blood flow) and acid production was found to obtain under all circumstances of accelerated or depressed activity. Vigorous contractions, while they did not invariably accompany redness of the mucosa, never occurred in the presence of pallor.

CONCLUSIONS

1. Studies have been pursued on a subject with a large gastric fistula whose peculiar defect provided the opportunity to collect at intervals the total volume of secretion in the stomach.

2. The rate of acid production in the stomach was estimated under varying conditions with reference to volume of secretion and concentration of acid. The results were expressed in terms of parietal cell output.

3. Increased parietal cell output was always associated with hyperemia of the gastric mucosa. Hyperaemia also accompanied increased motor activity.

4. On the basis of data gathered from these studies, certain generalizations are possible with regard to the interpretation of the findings on routine gastric analyses performed on patients.

a. When an unusually large volume of gastric juice of high titratable acidity accumulates in an unobstructed stomach during a specified interval, it is safe to assume that the mucous membrane is relatively red and that no vigorous contractions are taking place in the stomach.

b. When a small volume of gastric juice of very high titratable acidity is obtained under similar circumstances, it is likely that the mucous membrane is relatively engorged with blood and that, in addition, especially vigorous contractions are taking place.

c. Low acid values in the presence of small volumes of gastric juice strongly suggest that the gastric mucosa is relatively pale and that no vigorous contractions are taking place.

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PHOSPHORUS DEPOSITION IN THE EGG AS MEASURED WITH RADIOACTIVE PHOSPHORUS

F. W. LORENZ, I. PERLMAN AND I. L. CHAIKOFF

From the Division of Poultry Husbandry of the College of Agriculture, Davis, and the Division of Physiology of the Medical School, Berkeley, University of California

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The laying bird is characterized by an exceptionally high phosphorus turnover. Not only does phosphorus leave the bird through the usual channels of excretion but, in addition, large amounts are deposited in the egg. A single 60 gram egg may contain as much as 0.5 gram of phosphorus combined in various compounds.

The radioactive isotope of phosphorus provides a useful tool for tracing the passage of phosphorus into the egg. In the present study, the rate of appearance of labeled phosphorus was followed in the shell, membrane, albumen and yolk.

EXPERIMENTAL. Six laying White Leghorn hens (Gallus domesticus) were maintained in individual cages equipped with glass dropping trays. Each bird received subcutaneously 1.5 cc. of an isotonic solution of radioactive NaH_2PO_4 containing 10 microcuries of P^{32}. At the same time 0.5 cc. of an emulsion of Sudan III, similar to that used by Warren and Conrad (1), was injected intravenously. Some of this fat-soluble dye was deposited at once in the developing ova, forming a shell 0.5 mm. thick, clearly marking the size of the yolk at the time the radioactive phosphorus was injected. Droppings were collected at 6, 12 and 24 hours after the injection and at 24-hour intervals thereafter. These droppings were air-dried, ground and ashed. Eggs were collected at hourly intervals. They were frozen and then cut through the center of the yolk. Diameters of colored rings were measured. Shell, membrane, albumen and yolk were separated. An aliquot of the yolk was stored in alcohol for isolation of phospholipids; the remainder of the yolk and other parts of the egg were ashed in the presence of nitric acid and magnesium nitrate. For the determination of phospholipid the samples were extracted and mounted in the manner previously described (2).

RESULTS. The early growth of the ovum is a slow process (1, 3). Several months are required for the ovum to attain a diameter of 6 or 7 mm.; during this time only white yolk is deposited. The final stage of yolk growth, which involves the deposition of yellow yolk, is a much more rapid process, requiring 7 to 10 days for completion. During the first part of this period the yolk diameter increases about 2 mm. per day, but during the last 3 or 4 days the daily increment in yolk diameter gradually decreases.

Within a few minutes after ovulation the yolk is picked up by the infundibulum of the oviduct. It traverses the magnum in 3 hours and is meanwhile surrounded with concentrated albumen (fig. 1). The egg obtains all of its albumen protein and about half of its water while traversing this structure. It

next enters the isthmus. It requires a little over an hour for its passage through here, and during this interval the egg receives its membranes. During its stay in the isthmus some water and crystalloids enter the albumen by osmosis through the membranes. The egg next enters the uterus, where it remains, on an average, 21 hours. During this interval the shell is deposited; additional water and salts are also added to the albumen during the early part of this interval.

Deposition of labeled phosphorus in egg shell. Labeled phosphorus was found in the shells of all eggs laid subsequent to P^{32} injection. The period of observation extended over an interval of 504 hours. One egg present in the uterus at the time of P^{32} administration was laid one hour later, and its shell already contained 0.15 per cent of the injected P^{32}. Since about 21 hours are required

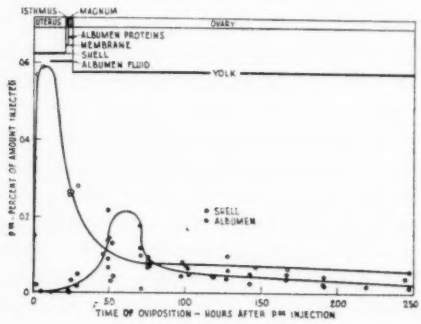

Fig. 1

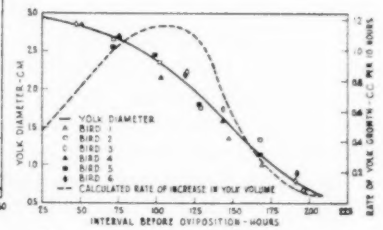

Fig. 2

Fig. 1. Labeled phosphorus deposition in albumen and shell. Each point represents the quantity formed in the whole albumen or shell of an egg laid at time indicated. The position of the egg in the bird's reproductive tract at the moment of P^{32} injection is shown at the top.

Fig. 2. Increase in yolk size. Solid line represents yolk diameter at time of P^{32} injection as measured by dye technique. Broken line shows rate of increase of yolk volume calculated from yolk diameter curve.

to form a shell, it is apparent that very little could have been added to the shell in the last hour. Three other eggs, laid, 2, 3 and 6 hours after injection, contained progressively larger amounts—namely, 0.35, 0.57 and 0.59 per cent respectively. The rapidity with which labeled phosphorus appeared suggests that blood phosphorus is immediately available for deposition in the shell.

The next eggs were laid 24 to 30 hours after the injection of labeled phosphorus. Shell formation was therefore not initiated in these eggs until 3 to 9 hours after the P^{32} injection, and so P^{32} was available during the entire period of shell formation. Yet these shells contained only 0.23 to 0.28 per cent of the injected P^{32}, amounts even less than that present in the shell of the egg laid only 2 hours after injection. Succeeding egg shells had even smaller amounts of phosphorus, but the quantity decreased only very slowly in shells of eggs that were laid after 50 hours after injection. In figure 1 values for shell P^{32} are plotted against the

interval between the injection and the time the egg was laid. Values for intervals up to 250 hours only are shown in figure 1. Radioactivity, however, was still perceptible in the shell of an egg laid as late as 504 hours.

As pointed out above, only those shells that were being actively formed at the time of the injection showed a high P^{32} content, whereas eggs that entered the uterus several hours later contained much smaller amounts of P^{32} in their shells. This indicates that the source of shell phosphorus is a rapidly exchanging entity, presumably inorganic phosphorus.

In contrast to these findings on egg shell, no significant radioactivity was detected in the shell membrane at any time.

Radiophosphorus deposition in the albumen. Since albumen protein is secreted while the egg is in the magnum and its deposition is completed about 22 hours before oviposition, protein-bound radiophosphorus could not have appeared in eggs laid less than 22 hours after P^{32} injection. Some crystalloidal P^{32} might, however, have been added to eggs laid as early as 12 hours after its administration. Nevertheless, no appreciable quantities were found in albumen of eggs laid during the first 24 hours (fig. 1).

Eggs laid during the 24–30 hour interval entered the magnum at a time when blood radiophosphorus was probably at a maximum as judged by P^{32} deposition in the shell. These eggs, however, did not contain the greatest amounts of albumen radiophosphorus. As shown in figure 1, the P^{32} content in the albumen of these eggs did not exceed 0.05 per cent, whereas eggs laid between 45 and 75 hours contained 2 to 4 times this amount. Albumen of succeeding eggs showed low levels of P^{32}, which continued to decrease throughout the period of observation. Radioactivity was still perceptible in the last egg that was analyzed; this egg was laid 504 hours after injection.

There is thus a marked dissimilarity in the deposition of phorphorus in shell and in albumen. The delayed deposition of P^{32} in the albumen suggests that a synthetic process precedes the deposition of phosphorus-containing compounds. Since at least one albumen protein, namely, ovalbumin, is known to contain phosphorus (4), the delay is not unlikely due to the incorporation of phosphorus into this or other protein before their deposition in the albumen of the egg.

Radiophosphorus deposition in egg yolk. Yolks of eggs laid less than 24 hours after injection contained only traces (up to 0.0045 per cent) of the administered P^{32}. Since an egg spends 24 to 25 hours in the oviduct after ovulation, these yolks had already completed their growth and had left the ovary at the time the injection was made. Consequently, no P^{32} was to be expected except for such small amounts as may have passed through the vitelline membrane by osmosis from the albumen. Two birds (2 and 5) laid eggs 30 hours after injection, and yolks of these eggs each contained only 0.010 per cent of the administered phosphorus. The next eggs obtained were from bird 3 at 47 hours, bird 4 at 50 hours, and bird 1 at 51 hours. Yolks from these eggs had been in contact with the ovarian vascular system for 22 to 26 hours after the injection of P^{32}; these contained appreciably larger amounts of the labeled phosphorus (0.275, 0.985 and 0.605 per cent respectively).

The quantity of P^{32} in yolks of succeeding eggs laid by each bird increased until a maximum was reached; the maxima for all birds were observed between 98.5 hours (bird 5) and 146.8 hours (bird 1) after the injection of P^{32}.[1] The maximum quantities of radioactive phosphorus varied from 1.19 per cent for bird 89 to 2.48 per cent for bird 5. Following maximum deposition, the amounts of radiophosphorus in succeeding yolks decreased abruptly at first and more gradually thereafter.

A simple examination of such data affords little information about the factors involved in the deposition of a particular substance in the egg yolk. One of the complicating factors is the rapid change in the amount of P^{32} available for yolk formation. Thus, yolks proceeding through a particular phase of growth several days after the P^{32} is administered will necessarily draw upon phosphorus of lower specific activity than those yolks passing through the same growth phase at an earlier time. Moreover, in view of the marked differences in the types of P compounds occurring in yolk, it would be desirable to consider the deposition of individual phosphorus compounds rather than total phosphorus.

In the present study attempts have been made to resolve these difficulties as follows: 1. Egg yolk phosphorus was separated into two components: phospholipid and non-phospholipid or "residual" phosphorus. This separation, of course, merely divides the phosphorus present into two main groups. 2. From theoretical considerations, attempts were made to calculate a series of yolk P^{32} values that agreed closely with the experimentally observed values of P^{32} in each yolk fraction. The best agreement was obtained when values were calculated on the assumption that the amount of the P^{32} fraction in any small increment of newly deposited yolk is proportional to the concentration of that P^{32} fraction or its precursor in the circulation at that time. The theoretical expression thus obtained (see addendum) permitted calculation of a single series of values for each phosphorus fraction that agreed well with the distribution of experimental values in successive yolks from the same bird (figs. 4 and 5). The theoretical expression differed from the experimental values by a factor which, though constant for all values of a single bird, differed for different birds.

Radiophosphorus excretion. In marked contrast to the patterns of P^{32} deposition observed in different parts of the egg, excretion of radiophosphorus in the droppings (mixed urine and feces) showed a marked irregularity. The hourly excretion of radiophosphorus in the droppings of individual birds is plotted in figure 6. In each histogram, the abscissa represents the period during which the sample was collected, and the ordinate represents the average hourly excretion during that period. The arrows represent periods during which egg shells were formed, and each point of the arrow shows the time the egg was laid. The four rapidly laying birds, 1, 2, 4 and 5, excreted radiophosphorus slowly and somewhat irregularly. Except for a somewhat higher excretion rate during the

[1] The maximum value for bird 6 was 0.651 per cent in an egg laid 76 hours after injection; this egg, however, was followed by a double-yolked egg at 118.8 hours, the two yolks of which contained 1.49 per cent. To avoid complicating factors this latter egg has been eliminated from discussion.

first day, variations were not great and seemed to occur at random. By contrast, however, the two slowly laying birds, 3 and 6, showed periods of increased excretion that coincided with periods of shell formation.

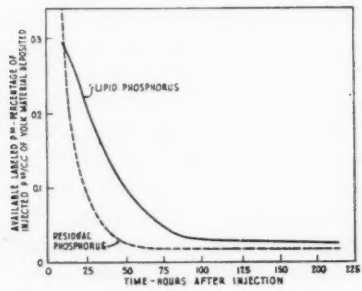

Fig. 3

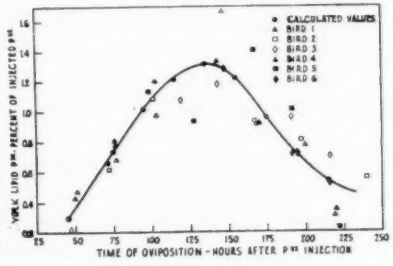

Fig. 4

Fig. 3. Availability of P^{32} for lipid phosphorus and residual phosphorus of yolk. Curves chosen by trial and error to fit observed P^{32} data.

Fig. 4. Labeled phospholipid content of yolks of eggs laid after P^{32} injection. The curve was calculated from equation (1). Each point represents total radiophospholipid in one yolk, adjusted so that value for the yolks from each bird have the same mean as corresponding points on calculated curve. (Points from each bird are thus plotted as deviates from a single theoretical curve. To obtain actual values, multiply ordinates of curve by corresponding factor recorded in table 1 and add indicated deviate. For example, to obtain actual value for bird 41 at 103 hours, multiply 1.10 (read from curve) by 0.694 (from table 1) and subtract 0.13 (which is the difference between the plotted point and 1.10).)

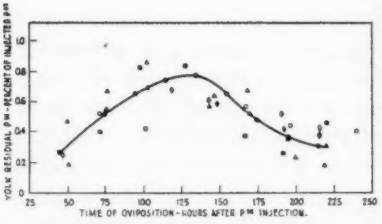

Fig. 5

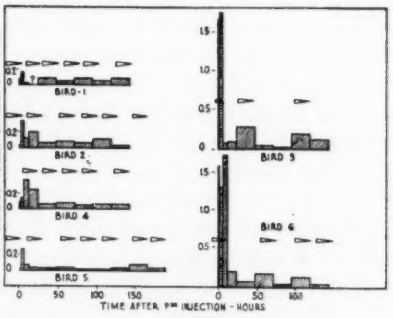

Fig. 6

Fig. 5. Residual radiophosphorus content of yolks of eggs laid after P^{32} injection. Calculated curves and experimental deviates plotted as in figure 4. For meaning of symbols see figure 4.

Fig. 6. Rate of labeled phosphorus excretion in the droppings. Arrows point to time of oviposition and extend over period egg remained in uterus.

These results are particularly interesting in view of the findings of R. H. Common (6, 7, 8), who reported that phosphorus excretion in the droppings increased during egg-shell formation in birds on a diet containing 0.67 per cent

calcium or less, but not as much as 2.21 per cent calcium. The birds in the present experiment were maintained on a diet containing 1.21 per cent calcium. The pattern of P^{32} excretion in only 2 of the 6 birds studied was similar to that demonstrated by Common for total phosphorus. These results are not necessarily contradictory, however, for the two birds that followed Common's pattern were slow-laying, as were all the birds studied by him.

DISCUSSION. Although the mechanism proposed to account for the phosphorus deposition in yolk (namely, that the total amount present is an integral of the product of yolk-growth rate and phosphorus availability[2]) was found to fit the experimental data satisfactorily, one obvious discrepancy was left unresolved. Large individual differences among birds necessitated the use of different factors (Table 1) in order to compare the theoretical curves with experimental values. These factors (which correspond to k in equation 1) varied between 0.39 and 1.41 for the radiophospholipid curve and between 0.57 and 1.27 for the residual P^{32} curve. The underlying nature of such factors deserves inquiry. The two lowest residual phosphorus factors were observed for birds 3 and 6, and the lowest and third lowest phospholipid factors were also

TABLE 1

k values for equation (1)

BIRD	PHOSPHOLIPID P^{32}	RESIDUAL P^{32}
1	0.694	1.274
2	1.367	0.959
3	0.793	0.574
4	1.166	1.142
5	1.414	1.135
6	0.391	0.634

found in these birds. These 2 birds were the slow layers that excreted large amounts of phosphorus in their droppings.

This relation between rate of laying, phosphorus excretion, and the above k values offers an explanation of the low factors of birds 89 and 92 and thereby extends Common's theory. Common explained his results by postulating a withdrawal of calcium phosphate from the skeleton for shell formation, the calcium being used for the shell and the extra phosphorus excreted. Evidence confirming this view was offered by Feinberg, Hughes and Scott (9), who demonstrated an increased blood inorganic phosphorus during the time of shell formation (from 4.2 mgm. per cent immediately after ovulation to 7.5 mgm. per cent after the egg reached the uterus). The phosphorus availability curves in figure 3 then must be partly dependent on the presence or absence of eggs in the uterus, and should show corresponding fluctuations. No attempt was made to include these fluctuations in figure 3, and they probably account for part of the experimental error (equation 4), although the method of treatment was such as to minimize errors due to such fluctuations. A gross difference

[2] See addendum.

between the two slow-laying and the four rapid-laying birds, however, cannot be neglected. In the former, shells were being produced less than 30 per cent of the time during the first 100 hours after injection of P^{32}, and in the latter about 80 per cent of the time. This difference must have been responsible for a large gross difference in the phosphorus-availability curves.

Shell formation, as already noted, presumably calls forth calcium and phosphorus from bone. The ratio of calcium to phosphorus in shell is much higher than in bone; obviously, therefore, the extra phosphorus must be disposed of elsewhere than in the shell. In the fast-laying birds, the rapidly growing ova have access to this extra phosphorus. The ovaries of the slow-laying birds, by contrast, probably contained relatively few rapidly growing yolks which could take up only a small portion of the extra blood phosphorus when made available. Consequently, in conformity with the pattern described by Common, a large portion of this extra phosphorus appeared in the droppings of the slow-laying birds as "extra" excretion during shell formation.

In the preceding discussion the assumption has been made that the availability curves (fig. 3) bear a close relation to the true plasma labeled phosphorus curves. Such an assumption will be valid only if the action of the follicular membrane does not change during the period of yolk growth considered here, in respect to any selective action it may exert on phosphorus-containing compounds supplied to it by the plasma. The data available do not permit an estimate of the shape of the portions of the availability curves during the first ten hours after injection. Certain general properties of the curves in this region are obvious, however, from the shapes of the curves as drawn. Both curves must have a maximum within ten hours after the injection, and the maximum for the residual phosphorus curve probably occurs earlier and is higher than that for the phospholipid curve.

Data on the deposition of radiophosphorus in the egg have appeared in three previous communications (10, 11, 12). Entenman et al. (10) injected P^{32} and determined labeled phospholipid deposition in growing ova during an interval of six hours after injection. Radiophospholipid deposited in smaller ova, which weighed between 0.69 and 5.8 grams, varied from 0.0023 to 0.058 per cent, increasing regularly with increasing size of the ovum. In larger ova this relation no longer held; the maximum value, 0.068 per cent, was found in a 10.7 gram ovum, and ova larger than this had somewhat smaller quantities of radiophospholipid. A similar distribution of values, although with correspondingly larger total quantities, was obtained from ova of birds killed twelve hours after P^{32} injection. Such values fit well with expected deposition rates that might be predicted from the yolk growth curve in figure 2. This correspondence may be extended by the two values obtained in the present investigation for yolks in which radiophospholipid was deposited for five to six hours after P^{32} injection at the end of their growth period. These yolks received only 0.010 per cent of the injected P^{32} as phospholipid, an amount very much less than that found in the largest ovum taken from the ovary.

Hevesy and Hahn (11) measured radiophospholipid deposition in the smaller

ova of a single hen killed 28 hours and two ova from another bird killed five hours after P^{32} injection. The results, expressed as "specific activity," cannot be compared directly with the foregoing, but nevertheless demonstrate the same increase with increasing size of smaller ova. These investigators also measured P^{32} distribution in five eggs laid subsequent to P^{32} injection. All of their values were appreciably lower than those reported in the present paper at comparable intervals. From the low activities found in the albumen of the first egg laid (reported variously as 4.5 hours and as 0.17 day) after injection and in an egg removed from the oviduct of another bird 5 hours after injection, they postulate a delay in phosphorus-compound formation similar to that noted here. The 4.5 hour egg, of course, must have been in the uterus with its shell over half formed at the time of injection; consequently the P^{32} found must have diffused through both shell and membrane with the last bit of fluid that is added to the egg in the uterus. The second and third eggs measured by these investigators were laid 24 and 72 hours after injection, and therefore they missed the deposition peak recorded here.

The radioactive phosphorus used in this investigation was supplied by members of the Radiation Laboratory under the direction of Prof. E. O. Lawrence, to whom our thanks are due.

SUMMARY

1. Injected phosphorus makes its appearance rapidly in the shells of eggs. Largest amounts were found in shells being formed at the time of P^{32} injection.

2. The deposition of labeled phosphorus in albumen was delayed. Maximum deposition of P^{32} occurred in eggs in which albumen was actively secreted 24–54 hours after the P^{32} injection.

3. Administered P^{32} makes its appearance rapidly in the phospholipid and other phosphorus compounds of the yolk. The amount of P^{32} deposited in these fractions in the yolk could be accounted for by an integral function of the two variables, yolk growth rate and P^{32} availability, during the corresponding period of new formation; values so calculated agreed well with observed values. The availability curves for yolk phosphorus and yolk phospholipid have been roughtly defined.

4. A faster deposition of P^{32} was observed in yolks of fast-laying than in slow-laying birds.

5. Excretion of injected P^{32} is shown to depend upon *1*, rate of egg production, and *2*, whether shell formation is in progress.

Addendum. The deposition of a P^{32} fraction in yolk may be considered to take the form:

$$y = k \int_{t_i}^{t_o} x \frac{dv}{dt} dt \qquad (1)$$

where y is the total quantity of the substance containing P^{32} found in any one egg yolk, x a function of the amount of P^{32} available for the formation of substance y at time t, and $\frac{dv}{dt}$ the rate of growth of the yolk. The integration is carried out over the interval from t_i,

the time of injection, to t_o, the time of ovulation. Neither x nor $\frac{dv}{dt}$ is known analytically, but

$$\frac{\Delta v}{\Delta t} = f(t) \tag{2}$$

can be obtained graphically from the data shown in figure 2. The general form of the curve

$$x = \phi(t) \tag{3}$$

can be approximated from theoretical considerations. With an experimental curve for the one function (equation 2) and a hypothetical curve for equation 3, it should be possible to perform the integration of equation 1 graphically, to obtain a curve that corresponds closely to the experimental curve for the egg yolk phosphorus component (y), and to test the fit statistically.

To obtain the curve for $\frac{\Delta v}{\Delta t} = f(t)$, use was made of rings obtained in the yolks by Sudan III injection at the time of radiophosphorus administration. The yolk diameters at the time of injection so obtained were plotted against the interval between injection and oviposition (fig. 2) and a mean curve was drawn. From values read off this mean curve, volume increments were calculated by assuming that yolks are perfect spheres. Ten-hour volume increments were plotted against time before oviposition (fig. 2).

To obtain an approximation to the curve $x = \phi(t)$ it was assumed that the labeled phosphorus, after reaching an early maximum in the bloodstream by rapid absorption from subcutaneous tissues, is lost from the blood at a rate proportional to the amount present. This relation could hold, however, only in the earlier stages; instead of approaching zero asymptotically, the available phosphorus should remain at a low but very slowly decreasing level for a long time, since labeled phosphorus deposited earlier in the skeleton gradually re-enters the circulation. Cook, Scott and Abelson (5) have shown that chicken bones contained appreciable amounts of radiophosphorus 75 days after injection. Such a curve should bear a close similarity to the experimental curve of radiophosphorus deposition in shells, since the amount of P^{32} deposited in the shells responds most readily to the level of circulating P^{32}. Starting with this curve as a first approximation to the P^{32} availability curve, two curves were finally chosen by trial and error to represent availability of P^{32} for phospholipid and residual P. These curves are shown in figure 3.

A theoretical curve for deposition of a phosphorus compound could then be obtained by integrating equation (1) graphically for a sufficient number of points. Nine such points (each representing a hypothetical yolk) were chosen to define the curve between oviposition intervals of 45 to 215 hours after injection of P^{32}. Each point was obtained by summing products of values read from figure 2 ($\frac{\Delta v}{\Delta t}$ values) and from figure 3 (x values) at corresponding 10-hour intervals between the time of injection and of ovulation of the hypothetical yolk. To be sure, the points do not define a unique curve, but rather a series of curves, since the nature of k (equation 1) has not been considered. As stated above, k differs for different birds. Thus to compare this curve with the experimental data the graphically integrated function $\Sigma(x\frac{\Delta v}{\Delta t})$ must be multiplied by a different k for each bird. Such curves were drawn for each bird, and the deviates (d_i) of each experimental point were thus obtained from the individual theoretical curve for that bird (table 1). To save space, however, a single theoretical curve only was plotted for each fraction (phospholipid-P^{32} in fig. 4; residual-P^{32} in fig. 5), with the average value of k. How well the experimental points fit their individual theoretical curves is illustrated by plotting their deviates (d_i) on the mean theoretical curve.

How well the experimental points fit the theoretical curves was tested by calculation of t values for various regions; a significant t value would indicate a significant departure of the points from the theoretical curve and, hence, a poor fit in that region. Deviates from the individual theoretical curves as previously defined were used to calculate t for each region by the formula:

$$t = \frac{\sum^{n} d_i}{\sqrt{n}} \sqrt{\frac{N - 6}{\sum^{N} d_i^2}} \qquad (4)$$

where the values of d_i of all birds in the region under test are summed. N is the total number of observed values and n the number of observations in this region.

For test the curves were divided into four approximately equal segments; the t value for each region was non-significant. In one region only (100–149 hrs. on the residual phosphorus curve) was the probability as low as 0.1; in all other instances the probability varied from 0.4 to greater than 0.9. This test becomes more sensitive as shorter and shorter regions are tested, and may even be applied to individual points. In this test only a single point on the residual phosphorus curve and only 2 points on the phospholipid curve had t values large enough to be assigned a probability of 0.05, the accepted minimum criterion of significance. Further, the deviates can be shown to be normally distributed. The theoretical curves, therefore, may be said to fit the experimental points adequately, and the hypothetical mechanism of radiophosphorus deposition (equation 1) may serve as a first approximation, at least, to the actual mechanism.

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OXYGEN CONSUMPTION IN VITAMIN E DEFICIENCY[1]

HANS KAUNITZ AND ALWIN M. PAPPENHEIMER

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, N. Y.

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Vitamin E deficiency leads to extensive degeneration of the skeletal muscles in several species of mammals. Previous studies in rabbits, guinea pigs, older rats, and hamsters by Victor, Madsen, Friedman and Mattill, and Houchin have shown an increase in the *in vitro* O_2 consumption of the muscles (1, 2, 3, 4). These studies, however, have been carried out in animals in which dystrophic changes in the muscles had taken place and in which the interpretation of the results may have been complicated by the presence of inflammatory and regenerating cells or by the replacement of the muscle fibers by fat or connective tissue. Moreover, in the experiments of Victor, Madsen and Friedman and Mattill, the data were compared with those obtained from animals on a stock diet rather than with those on an experimental one supplemented by alpha-tocopherol.

It seemed important, therefore, to determine whether a rise in the rate of O_2 consumption of the rat muscle *in vitro* occurred also in the absence of morphologic changes, and whether administration of alpha-tocopherol would prevent the increase.

We have also included in our studies muscles from a species in which the manifestations of vitamin E deficiency are chiefly limited to the central nervous system. This is the case in chicks (5). With the vitamin E deficient diet used in this laboratory (diet 108), the skeletal muscle is rarely affected and only in animals showing the "alimentary exudative diathesis" described by Dam, Glavind, Bernth and Hagens (6).

Information as to the O_2 consumption of whole animals kept on a vitamin E deficient diet is scant. Wood and Hines (7) reported 116 examinations on 10 guinea pigs on a vitamin E deficient diet which were examined at weekly intervals over a period of 2 to $3\frac{1}{2}$ months. No significant difference between these animals and controls on a stock diet could be discovered when the values were expressed in cubic centimeters of O_2 per hour per square meter.

Telford, Emerson and Evans (8) found in preliminary studies on 4 vitamin E low and 4 normal rats (whose age is not stated) no significant differences. The average figure for the vitamin E deficient animals was "slightly higher" than that for the normal ones.

Biddulph and Meyer (9) examined the O_2 uptake of rats which had been kept on a vitamin E deficient diet after the age of one month. They found the O_2 consumption in vitamin E deficient animals significantly higher than that of rats on a stock diet. This difference, however, did not become evident until after six months on the experimental diet, and was associated with enlargement

[1] Aided by a grant from the John and Mary R. Markle Foundation.

of the thyroid gland. Addition of KI to their diet decreased both the oxygen consumption and the thyroid weight, but the administration of wheat germ oil alone had the same effect.

These studies and those of the other workers cited did not include observations on young rats during the acute phase of muscle degeneration when the lesions are most severe, nor upon the possible effect of the administration of alpha-tocopherol. It seemed important to find out whether the increase in O_2 consumption noted in excised muscle might be reflected in an increased O_2 uptake of the living animals and, if so, to what extent this could be prevented by the administration of tocopherol.

I. O_2 CONSUMPTION OF RAT AND CHICKEN MUSCLE IN VITRO. 1. *Rats.* The mother animals were maintained from the time of weaning on a vitamin E deficient diet which consisted of: casein (commercial), 320 grams; corn starch, 400 grams; salts (Hawk and Oser), 40 grams; yeast (baker's dried), 100 grams; lard, 220 grams; cod liver oil, 20 grams. During lactation, 100 grams of yeast were added to the diet.

When mating was positive, the litter was assured by protecting the mother with 50 drops of wheat germ oil given within five days after mating. Under these conditions about 90 per cent of the offspring develop lesions (10).

The young in which protection was desired received one drop of sesame oil, containing one milligram of synthetic dl-alpha-tocopherol acetate (Hoffmann-La Roche)[2] by mouth on the 15th day. They were separated for a certain time from the unprotected animals in order to make the alpha-tocopherol inaccessible to them. Muscles from rats maintained on a stock diet (Rockland pellets) were also studied for comparison.[3]

Chicks. These were placed 3 or 4 days after hatching upon the standard encephalomalacia-producing diet 108 (11). Dl-alpha-tocopherol acetate was given in daily doses of 0.2 mgm. in gelatin capsules. Control chicks were given commercial mash (Ful-o-Pep Egg Breeder Mash made by the Quaker Oats Company).

The O_2 consumption was measured in Fenn's constant pressure differential manometer (12). The modification described by Victor and Potter (13) was followed in this work. QO_2 is expressed in millimeters^3 per gram wet weight in 1 minute. The water bath in rat experiments was kept at 37°C. and in the chick experiments at 39°C.

The animals were killed by decapitation. The flexor muscles of the hind legs and the pectoralis muscles were chosen for study in rats; in chicks, only pectoralis muscle was used. The pieces were sliced into thin strips with a safety razor blade while still *in situ*. About 15 minutes elapsed between the death of the animal and the immersion of the bottles with the muscle fragments

[2] We are greatly indebted to Dr. R. D. Shaner of the Hoffmann-La Roche Company for supplying dl-alpha tocopherol acetate.

[3] The Rockland diet is stated to contain 3.2 mgm. per cent of alpha-tocopherol as determined by rat bio-assay. We are indebted to Dr. Charles A. Slanetz of the Department of Animal Care for this information.

in the water bath. For each examination the average of at least two control bottles was taken. In most of the experiments litter-mates were examined on the same day.

Histological sections of the muscles were made from all animals and in addition, the muscle pieces used for the determination of the oxygen consumption were fixed in 10 per cent formalin at the end of the experiment and examined histologically.

EXPERIMENTS. *Rats.* The individual data on leg muscles of rats, together with the mean, are shown in figure 1, and the results of all experiments are summarized in table 1.

The mean O_2 consumption of the leg muscle from the vitamin E deficient group during the first hour is significantly greater than that from the group which received a single dose of dl-alpha-tocopherol acetate given between the

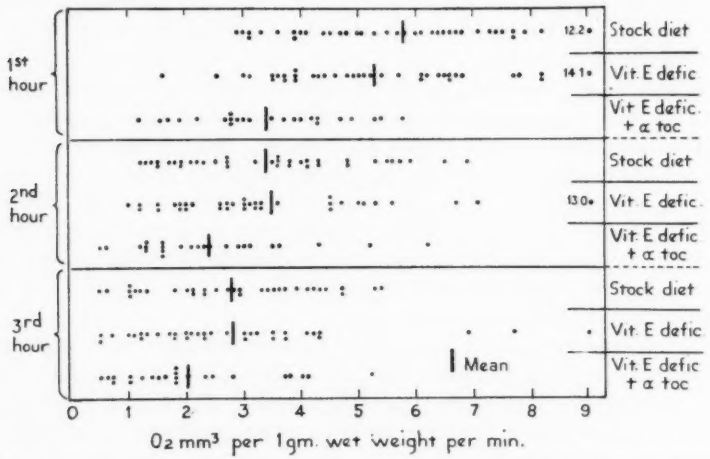

Fig. 1. O_2 consumption of excised rat muscle from rats on experimental diets

13th and 15th days. The difference is also significant during the second hour, but tends to disappear in the third hour after the beginning of the experiment.

Comparing the vitamin E deficient animals with those on stock diet, presumably containing an abundance of vitamin E, it is surprising that there is no statistically significant difference in the O_2 consumption. Stock animals which received a supplementary single dose of 1 mgm. of dl-tocopherol acetate showed practically the same values. While we are unable to account for this discrepancy, the difference in the composition of the stock diet and the simplified experimental diet makes it possible that other factors than the vitamin E content may be involved.

The data obtained from the pectoralis muscle show differences in the same direction between the muscles of the vitamin E deficient group and those which had received a protective dose of alpha-tocopherol. The difference is manifest only during the first hour and is less pronounced than with the leg muscles.

Table 2 shows that there is no statistically significant difference between the O_2 consumption of muscles showing dystrophic lesions and those which were histologically normal.

In order to ascertain whether other tissues than the skeletal muscle show an increased O_2 consumption in vitamin E deficiency, determinations were made on the liver of 19 vitamin E deficient, and 17 tocopherol treated rats. No significant difference was found.

Chicks. A summary of our determinations is given in table 3. The experiments were run in two series—one in the spring of 1941 and the other in Novem-

TABLE 1

In vitro O_2 consumption of rat muscles

(Cubic millimeters per gram wet weight per minute)

TISSUE	AGE RANGE	DIET	NO. OBS.	FIRST HOUR			SECOND HOUR			THIRD HOUR		
				Q/O_2	Range	σ	Q/O_2	Range	σ	Q/O_2	Range	σ
	days											
Hind leg muscle	19–26	Stock	38	6.8	3.9–12.2	1.83	4.4	2.2– 7.9	1.56	1.56	1.5– 6.4	1.36
Hind leg muscle	19–20	Stock + single dose of 1 mgm. α toco-	13	6.4	3.3– 8.4	1.49	4.8	2.0– 6.8	1.28	4.3	1.2– 6.5	1.46
Hind leg muscle	14–23	Vitamin E deficient	37	6.3	2.6–14.1	2.10	4.5	2.0–13.0	2.19	3.8	1.5–10.0	1.92
Hind leg muscle	14–23	Vitamin E deficient + single dose of 1 mgm. α tocopherol	24	4.4	2.2– 6.8	1.29	3.4	1.5– 7.2	1.36	3.0	1.5– 6.2	1.31
Pecto-ralis muscle	14–23	Vitamin E deficient	23	5.7	2.8– 8.8	1.46	3.8	2.2– 6.5	1.27	3.0	1.5– 6.4	1.36
Pecto-ralis muscle	14–23	Vitamin E deficient + single dose of 1 mgm. α tocopherol	24	4.9	2.9– 8.3	1.15	3.8	2.1– 5.7	1.10	3.2	1.5– 5.9	1.15

ber and December of the same year. There was no indication of a seasonal difference, and the data show close agreement.[4]

DISCUSSION. The above findings confirm the previous observations of Victor,

[4] Dr. Joseph Victor and Dr. L. L. Madsen very kindly put at our disposal unpublished data on O_2 consumption of chick muscle. Their determinations were carried out in this laboratory several years ago, but their data are not strictly comparable to ours, inasmuch as the metabolism of the muscle of vitamin E deficient chicks in their experiments was compared with that of chicks on a simplified complete diet, but not on diet 108 supplemented by alpha-tocopherol. Both these workers, however, found a higher oxygen consumption on the vitamin E deficient diet—a result in line with our own observation.

Madsen, Friedman and Mattill and Houchin as regards the increased uptake of oxygen in dystrophic muscles. Our experiments, however, bring out the significant point that this metabolic change may be detected in rats even when the E deficient diet has brought about no anatomical changes in the muscle, and in chicks in which the skeletal muscle is unaffected. After dystrophic lesions are established, there is always a reaction in which wandering cells and young con-

TABLE 2

In vitro O_2 consumption of rat muscle showing dystrophic lesions on a vitamin E deficient diet

(Cubic millimeter per gram wet weight per minute)

LEG MUSCLE			PECTORALIS MUSCLE		
First hour	Second hour	Third hour	First hour	Second hour	Third hour
7.7	5.5	4.5	5.0	2.7	2.1
4.6	2.8	2.3	4.3	2.2	1.5
9.2	8.1	8.7	8.8	6.4	6.4
5.9	4.0	3.0	5.0	3.9	2.6
5.6	3.7	3.3	5.3	2.9	1.7
7.1	6.3	4.7	7.1	5.3	5.2
6.1	5.5	5.2			
5.4	2.5	3.2			
4.1	3.8	4.0			
4.7	3.1	2.0			
2.6	2.5	2.2			
Mean..5.7	4.3	3.9	5.9	3.9	3.3
σ.....1.81	1.79	1.96	1.70	1.65	1.92

TABLE 3

In vitro O_2 consumption of chick pectoralis muscle

(Cubic millimeter per gram wet weight per minute)

DIET	NO. OBS.	FIRST HOUR			SECOND HOUR			THIRD HOUR		
		Mean	Range	σ	Mean	Range	σ	Mean	Range	σ
Stock.....	18	5.4	2.7–9.1	1.72	3.5	1.2–5.9	1.19	2.5	1.1–4.5	0.92
Vitamin E deficient.	27	6.1	2.8–9.9	1.12	4.2	2.4–7.0	1.22	3.7	2.2–5.8	0.92
Vitamin E deficient single dose of 1 mgm. α tocopherol	39	5.3	2.7–9.3	1.41	3.7	1.2–5.3	0.99	3.1	1.3–4.4	1.83

nective tissue cells are concerned. The total oxygen consumption under these conditions represents the composite activity of several tissue elements. In our material, excluding the 11 cases in which lesions had developed, the increased oxygen consumption must be referred to a change in the metabolic activity of the muscle fiber itself.

It is further apparent from our data that the administration of synthetic

alpha-tocopherol acetate in a dose of one milligram given on the 11th to 14th day after birth significantly lowers the oxygen consumption as compared with the untreated controls. Friedman and Mattill (3) have reported a few preliminary experiments on 5 months old female rats in which the administration of 5 mgm. of alpha-tocopherol acetate lowered the oxygen consumption after 24 and 72 hours respectively.[5]

An important question, which has not yet been investigated, is whether the increased oxygen consumption is specifically limited to skeletal muscle or whether it is a general effect on the tissues. The fact that the increased oxygen consumption was not found in the liver suggests a characteristic effect on the muscle metabolism. Further studies, however, on other tissues would be needed to demonstrate this specificity.

II. TOTAL OXYGEN CONSUMPTION. Three groups of rats were used; the first received stock diet, the second the vitamin E deficient diet, the third the vitamin E deficient diet supplemented by a single dose of 1 milligram dl-alpha-tocopherol acetate on the 15th day of life. The tocopherol was given by mouth in a drop of sesame oil.

The animals were placed in the apparatus after a fasting period of one hour; the tests were performed about one hour later. They were purposely not fasted over a longer period because it was necessary to examine the young ones daily or every other day.

The O_2 consumption was determined in a closed circuit apparatus, the construction of which is shown in fig. 2 a, b, c. The apparatus is a modification of that described by Rappaport and Gottdenker (14). Single tests of 5 to 10 minutes' duration can be carried out with sufficient accuracy; in fact, the short duration of a single experiment is an advantage for the determination of the O_2 consumption of small animals, inasmuch as they can be examined in periods of complete quietness.[6]

After the apparatus is adjusted and tested, the actual procedure is as follows. The syringe is put into motion; the animal is placed in the container; care must be taken that the three-way stopcock permits free communication between the outside air and the apparatus, so that the O_2 consumed by the animal in the preliminary period can be instantly replaced. After about 30 minutes, the float of the spirometer is elevated and the three-way stopcock is put into a position in which it connects the spirometer with the apparatus and closes off the outside air. Several curves are taken, and only entirely straight ones, indicating the absence of movements of the animal, are computed. The lowest value (not the

[5] Since the completion of these experiments, there has appeared a paper by Houchin and Mattill (Proc. Soc. Exper. Biol. and Med. **50:** 216, 1942), demonstrating an increased *in vitro* O_2 consumption of dystrophic hamster and rabbit muscle. The direct addition of water soluble alpha-tocopherol phosphate to the Locke-Ringer solution reduced the O_2 uptake by 35.7 per cent and 41.3 per cent—figures which roughly correspond to the difference found by us between vitamin E deficient rat muscle and muscle from animals which had received a supplementary dose of tocopherol before death.

[6] Because of the need to economize space, details as to calibration and testing of apparatus are omitted.

average) is eventually used, as it most closely expresses the resting metabolism.

Measurements were made at a temperature of 29°C. and the rats were permanently maintained at a temperature of 25 to 29°C. in order to obtain stable and comparable results (Benedict and Macleod, 15). The rats were weighed immediately after the test.

The determinations began on the 10–14th day of life and were carried out on the same animal during the first 3 weeks, daily or on every other day, later once a week. Litter-mates were usually examined on the same day. In order to avoid diurnal variations (Horst, Mendel and Benedict, 16), tests were performed only between 10 a.m. and 4 p.m.

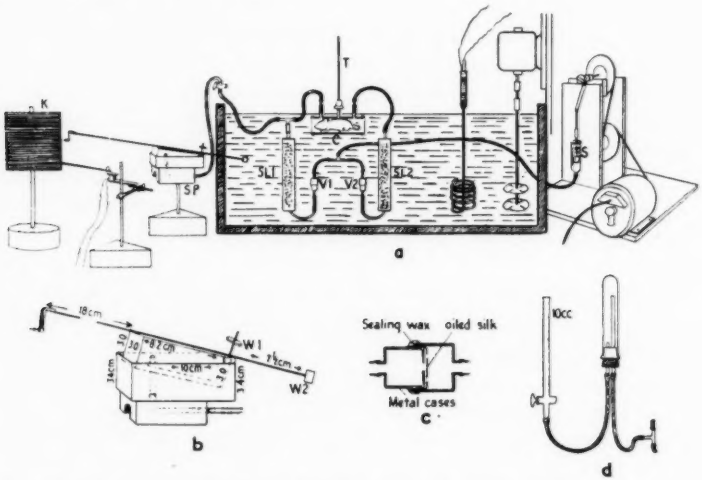

Fig. 2a. Closed circuit apparatus. *C*, animal container, dimensions 20 x 8 cm., closed by threaded glass windows. *SL 1* and *SL 2*, soda lime containers. V_1 and V_2, valves. *T*, thermometer. *S*, syringe, 40 strokes per minute. *SP*, spirometer. *K*, kymograph and timer.

Fig. 2b. Spirometer. W_1 and W_2, running counter-weights

Fig. 2c. Valve

Fig. 2d. Calibration apparatus

EXPERIMENTS. Data on the O_2 uptake expressed in cubic centimeter per 1000 grams per minute in relation to the age of the rats are presented in table 4 and figure 3. The figures obtained on stock diet are in close agreement with those of Davis and Hastings (17) on rats of corresponding ages, and with those of Griffith and Farris (18). We can also corroborate the statement of Davis and Hastings that the O_2 uptake of males and females does not differ up to the age of 4 months. Nevertheless, we have for other reasons excluded from our tables 4 and 5 results obtained on female rats.

Figure 3 demonstrates that the O_2 uptake per 1000 grams rat per minute between the ages of 15 and 150 days gradually decreases. This result also

TABLE 4

O_2 consumption of male rats in cubic centimeters per 1000 grams per minute according to age

AGE	STOCK DIET (11 ANIMALS)					VITAMIN E DEFICIENT DIET (17 ANIMALS)					VITAMIN E DEFICIENT DIET SUPPLEMENTED BY A SINGLE DOSE OF 1 MGM. TOCOPHEROL ON THE 15TH DAY (11 ANIMALS)				
	No. exper.	Mean O_2 cons.	Maxim. O_2 cons.	Minim. O_2 cons.	SE_m	No. exper.	Mean O_2 cons.	Maxim. O_2 cons.	Minim. O_2 cons.	SE_m	No. exper.	Mean O_2 cons.	Maxim. O_2 cons.	Minim. O_2 cons.	SE_m
days															
10–15	32	40.1	61	24	1.54	33	43.9	71	27	1.42	30	41.9	52	26	1.13
16–20	34	38.2	57	25	1.19	40	36.6	54	25	1.09	29	35.3	48	26	1.20
21–25	22	37.2	55	29	1.32	46	36.4	48	25	0.80	27	33.6	41	26	0.79
26–30	22	35.6	47	26	1.28	36	38.8	54	25	1.14	20	31.6	44	25	1.09
31–35	10	33.3	41	29	1.21	23	35.0	48	27	1.32	12	28.9	33	24	0.82
36–40	8	31.4	38	25	1.57	15	32.9	39	28	0.89	5	26.0	28	25	0.56
41–50	27	30.8	42	21	0.78	23	26.8	31	17	0.89	12	22.5	28	19	0.82
51–60	11	24.1	29	20	0.86	11	22.8	28	19	0.84	8	21.9	26	19	0.79
61–70	14	24.1	28	20	0.65	17	22.6	26	20	0.49	2	19.5	20	19	0.50
71–80	7	21.7	25	20	0.71	13	20.7	24	19	0.34	5	20.4	22	18	0.82
81–90	10	21.2	25	18	0.85	9	19.8	23	16	0.76	5	18.2	19	16	0.58
91–100	3	20.3	22	19	0.89	20	18.7	25	16	0.57	7	19.4	25	14	1.32
101–120	6	22.2	25	19	0.95	24	19.1	23	16	0.39	5	17.0	19	15	0.55
121–140	3	21.0	25	18	2.10	16	16.8	20	14	0.44	6	16.8	18	14	0.60
141–160						4	20.8	25	18	1.34					

TABLE 5

O_2 consumption of male rats in cc. per 1000 grams per minute according to weight

WEIGHT	STOCK DIET (11 ANIMALS)					VITAMIN E DEFICIENT DIET (17 ANIMALS)					VITAMIN E DEFICIENT DIET SUPPLEMENTED BY A SINGLE DOSE OF 1 MGM. TOCOPHEROL ON THE 15TH DAY (11 ANIMALS)				
	No. exper.	Mean O_2 cons.	Maxim. O_2 cons.	Minim. O_2 cons.	SE_m	No. exper.	Mean O_2 cons.	Maxim. O_2 cons.	Minim. O_2 cons.	SE_m	No. exper.	Mean O_2 cons.	Maxim. O_2 cons.	Minim. O_2 cons.	SE_m
grams															
10–20	24	40.3	58	27	1.90	24	43.7	71	30	1.81	9	42.0	52	26	2.80
21–30	35	40.1	55	25	1.26	56	39.7	54	25	0.92	31	42.5	50	34	0.76
31–40	22	36.1	43	29	0.95	48	37.5	53	25	0.94	21	34.4	41	30	0.75
41–50	19	37.1	48	29	1.25	22	36.9	46	30	0.96	18	33.1	44	26	1.22
51–60	10	31.9	41	26	1.38	15	33.6	46	24	1.28	14	31.0	36	27	0.71
61–70	6	33.2	38	28	1.62	10	33.3	39	28	1.20	7	29.7	33	26	1.03
71–80	3	29.3	34	25	2.07	7	32.0	36	27	1.36	3	27.0	28	26	0.91
81–90	5	33.6	42	29	2.32	6	33.7	39	29	1.50	8	29.3	33	25	1.08
91–100	7	30.9	34	27	0.94	4	29.8	31	28	0.63	4	29.2	33	25	1.75
101–120	14	29.6	33	25	0.93	9	26.9	29	25	0.45	7	27.7	29	26	0.54
121–140	10	29.4	39	25	1.58	15	25.8	31	19	0.89	5	25.0	27	22	1.09
141–160	8	25.0	29	21	0.93	7	25.0	29	22	1.19	8	22.1	28	19	1.30
161–180	6	23.7	28	20	1.23	8	23.1	28	17	1.21	1	20.0	20	20	—
181–200	17	23.6	28	20	0.52	8	20.5	24	16	1.16	6	21.8	24	20	0.63
201–225	8	21.8	25	18	0.88	14	20.9	24	17	0.43	4	21.8	22	21	0.33
226–250	9	20.1	22	18	0.54	19	19.6	24	15	0.50	4	19.7	21	19	0.65
251–275	3	21.0	24	19	1.26	21	18.8	23	16	0.43	8	19.5	25	16	0.98
276–300						22	19.0	25	14	0.51	3	17.8	19	16	0.91
301–325						11	18.9	25	16	0.78	3	16.3	18	14	1.21
326–350						5	16.8	19	15	0.66	4	16.8	19	14	1.30
351–375											6	17.3	18	17	0.22

corresponds to that obtained by Benedict and Macleod and by Davis and Hastings. The average values for the vitamin E deficient animals do not differ greatly from those of the ones on a stock diet, except for a dip in the curve between the ages of 15 to 30 days, the significance of which will be discussed later.

The vitamin E deficient animals whose diet was supplemented by a single dose of 1 mgm. tocopherol on the 15th day after birth showed a lower O_2 uptake from the 15th day to the 70th day. The statistical evaluation[7] indicated that the difference between the two groups became significant after the 20th day of life, and remained so until the 70th day except for the period of 51 to 60 days. *The single protective dose of 1 mgm. of alpha-tocopherol was therefore sufficient to change the O_2 consumption of the E deficient rats from weaning until sexual maturity.*

One might object that the weight of the animals was not taken into consideration. This objection seems justified inasmuch as the "protected" animals were usually heavier than unprotected ones, an observation that will be discussed in a later publication. We have therefore computed the O_2 consumption in cubic centimeters per 1000 grams per minute, according to the weight of the animals (table 5). The curve obtained by this presentation is similar to that in figure 3. The O_2 consumption again shows a gradual decrease; the curves of the stock rats and of the vitamin E deficient ones are similar; the values of the vitamin E deficient animals are higher than those of the protected ones over a range of from 30 to about 100 grams. The statistical evaluation again indicates a significant difference between individual points from 30 to 90 grams. The differences of the means are, however, slightly less pronounced than those in table 4 in which the O_2 uptake was computed according to the age.

Despite the overlapping of individual results, this also indicates that the single dose of alpha-tocopherol was sufficient to decrease the O_2 uptake of the rats from the time of weaning until sexual maturity.

The results have also been computed in relation to the surface area, which was calculated by Lee's formula, $S = 12.44\ W^{3/5}$, where S stands for the surface area in centimeters^2 and W for the weight in grams (19). The O_2 consumption in all three groups increased up to a body weight of about 100 grams and became constant thereafter. The initial increase is not due to the inapplicability of

[7] The data were statistically analyzed by finding the standard deviation, $\sigma = \sqrt{\frac{\Sigma^2}{n-1}}$ where x repreents the difference from the mean and n the number of experiments. The standard error of the mean, SE_m, was calculated by the formula, $SE = \frac{\sigma}{\sqrt{n}}$, and the standard error of the difference of the means by the formula, $SE_{m_{1-2}} = \sqrt{SE_{m1}^2 + SE_{m2}^2}$. If the difference of the means divided by SE_m showed a factor > 2, a significant difference could be assumed. The significance was even greater if subsequent points in the curve showed factors > 2.

For the help granted to us in the statistical analysis of our results, we are indebted to Prof. John W. Fertig of the De Lamar Institute of Public Health, Columbia University.

Lee's formula to small animals, since it has been shown by him to apply to animals of all weights. Again one finds that the curves of the vitamin E deficient and the stock rats are similar, whereas that of the "protected" animals is lower over the range of 30 to 90 grams; the statistical evaluation again confirms the significance of the differences.

An interesting feature of the O_2 consumption is observed during the period of the development and subsequent spontaneous regression of the muscle lesions. The animals usually develop general signs between the 18th and 22nd day and the survivors recover within 3 to 10 days after the onset. We observed signs of muscular disease in 13 of the 19 unprotected animals. In 8 of the 13 rats with signs, and in 2 of the 6 rats without signs,[5] we noted a considerable decrease of the O_2 consumption during the development of the disease (fig. 4). The low value disappeared when recovery began. In 3 cases in which the experiment was terminated by the death of the animal, the O_2 consumption showed a continual decline. Among 14 protected animals a similar curve was seen only once.

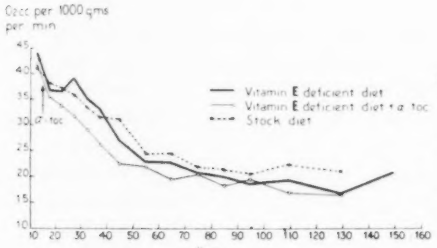

Fig. 3

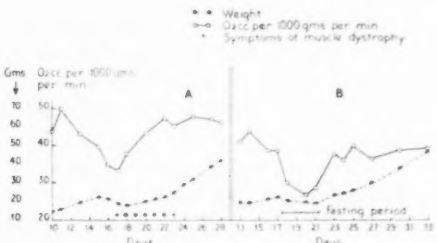

Fig. 4

Fig. 3. Average O_2 consumption of rats on experimental diets at different ages.

Fig. 4 A. Depression of O_2 consumption in a vitamin E deficient rat during dystrophic period. B. Depression of O_2 consumption produced by fasting in a normal rat.

In order to find out whether or not the described effect was specific for the vitamin E deficiency, or whether it was a consequence of the reduced nutrition and activity of the animals during the acute phase of the disease, comparable determinations were made on rats which were partially starved from the 18th to the 21st day after birth. They were the offspring of mothers maintained on a stock diet. We chose that time because it is the period during which the vitamin E deficient animals showed the dip in the curve. The animals were kept without food and water, but each day they were returned to their mother for two hours because it seemed probable that the vitamin E deficient animals secured at least some nourishment despite the disease. Figure 4 B shows the results obtained in an individual animal. The curve is very much like the ones obtained in the majority of the vitamin E deficient rats. The results of the tests cor-

[5] The occurrence of severe muscle lesions in the absence of clinical signs has been recorded by Pappenheimer (J. Mt. Sinai Hosp. **7:** 65, 1940) and by Telford, Emerson and Evans (Proc. Soc. Exper. Biol. and Med. **45:** 135, 1940).

respond with the experiments of Benedict and Macleod, who found that 24 hours' starvation reduces the O_2 uptake of young rats by 28 per cent. We performed the experiment on 7 rats from three different litters. The fasted rats were compared with their normal litter-mates. In the 7 fasted rats, the dip in the curve was observed 6 times; among the normal litter-mates it did not appear at all. These control tests indicate that the reduced O_2 uptake of the vitamin E deficient rats is a consequence of the starvation during the period of muscular dystrophy, rather than a specific effect of the vitamin deficiency.

In order to see whether the difference between the "protected" and the "unprotected" animals in the later stages of the experiment is a protracted effect of the starvation during the lactation period, the O_2 consumption of the partially starved animals on stock diet was compared with that of their unstarved litter-mates. One finds that there is a dip in the curve during the period of starvation, but later the curves up to 85 days are identical. The difference in O_2 consumption between the vitamin E deficient group and their protected litter-mates is therefore not due to transient inanition during the acute disease period.

Discussion. These determinations show that the total O_2 consumption of young rats on a vitamin E deficient diet, which have received a single dose of alpha-tocopherol on the 15th day, is lower than that of untreated litter-mates. The difference persists from the time of weaning until the onset of sexual maturity.

The living animal thus reacts in a fashion comparable to that of excised muscle *in vitro* and the question may be here discussed as to whether the increase in total O_2 consumption may be attributed to this change in the metabolism of the skeletal muscle. The average percentage increase based on weight in the living animal during the significant period is about 11.4 per cent, whereas that in the excised muscles was 30 per cent. The weight of the total skeletal muscular tissue is found by dissection to be about one-third of the body weight. If the increase of the O_2 consumption were wholly referable to the muscle tissue, and if the respiration of the muscle tissue *in vitro* were of the same order as in the living animal, one would expect an increase of roughly 10 per cent. Considering the many assumptions involved in such calculation, the order of magnitude of the change is not beyond what might be anticipated. That this increase in metabolism does not affect all the viscera is indicated by the fact that the *in vitro* O_2 consumption of the liver is unaltered in E deficient rats.

One may also raise the question as to a possible influence of the thyroid in stimulating the metabolism. Evidence gathered from the literature is conflicting. Singer (20) and Barrie (21) found hypoplastic glands in vitamin E deficient rats. Telford, Emerson and Evans (8) were unable to confirm this. Morgulis and Spencer (22) found no change in the relation of dry weight of the thyroid gland to body weight in dystrophic rabbits. On the other hand, Biddulph and Meyer (9) found a two-fold increase in the weight of the thyroid of male rats on vitamin E deficient diet. The addition of iodine to their vitamin E deficient diet reduced the thyroid weight and the increased metabolism to

normal, and the hypertrophy is ascribed to the low iodine content of their diet and drinking water.

In our experiments, a low iodine intake as a possible cause of thyroid hyperfunction can be positively excluded. On the basis of an average daily food consumption of 10 grams, our rats ingested 0.77 mgm. of iodine, calculated on the KI content of the Hawk-Oser salt mixture used in the experimental diet. We noted no difference in thyroid structure or weight in 3 unprotected and 2 protected rats kept for 5 months on vitamin E deficient diet.

A disturbing feature of our observations is that the stock rats showed an O_2 consumption which was much nearer to that of the vitamin E deficient than to that of the "protected" rats. We made a similar observation in our study of the metabolism of excised muscles. There also it was found that the average values in the E deficient animals matched those of the stock rats; the muscles of "protected" rats yielded considerably lower figures. Taken at their face value, the data would lead to the conclusion that the O_2 consumption of the vitamin E deficient rats is normal, that of the tocopherol treated rats, depressed. But wide difference in the composition of stock and experimental diets raises the question as to whether there may not have been other dietary factors than the vitamin E content which influenced the total metabolism. The possiblity that the stock diet used is low in its vitamin E content seems remote, since it maintains fertility in the breeding colony, prevents testicular atrophy and late muscular dystrophy, and contains 3.2 mgm. per cent of alpha-tocopherol by bioassay.

SUMMARY

1. The *in vitro* consumption of oxygen by the skeletal muscle of young rats born of vitamin E deficient mothers was found to be higher than that of littermates receiving a protective dose of 1 mgm. synthetic alpha-tocopherol acetate on the 15th day.

2. This difference may be noted in the absence of microscopic alterations in the muscle fibers.

3. The skeletal muscle of chicks on vitamin E deficient diet had a higher *in vitro* consumption of O_2 than that of chicks on a stock diet or on an E deficient diet supplemented by dl-alpha-tocopherol acetate.

4. No effect of vitamin E deficiency upon the *in vitro* uptake of rat liver could be detected.

5. The total O_2 consumption of rats on a stock diet, on a vitamin E deficient diet, and on a vitamin E deficient diet supplemented by a single dose of synthetic dl-alpha-tocopherol was determined over a period ranging from 11 days to 5 months of age.

6. Following a single dose of alpha-tocopherol given on the 15th day, the O_2 consumption was significantly lower than that of the untreated litter-mates. This difference persisted from the time of weaning (or recovery from the muscular dystrophy) until the 60th to the 70th day, corresponding to a weight range of about 30 to 100 grams.

7. During the development of the muscle disease (from about the 18th to 22nd day after birth), the O_2 consumption of the E deficient group was decreased; this effect seemed to be a consequence of the reduced nutrition and activity during that period, since it could be imitated in partially fasted rats of the same age. Partial starvation towards the end of the nursing period did not, however, lead to protracted changes in the O_2 consumption of rats on a stock diet.

8. The O_2 consumption of rats on a stock diet was found to be similar to that of the vitamin E deficient ones. No satisfactory explanation for this fact can be given.

We wish gratefully to acknowledge the skilled collaboration of Dr. Constantino Mignone, Fellow of the Rockefeller Foundation, in the first part of this work; and to thank Mrs. Thelma Stout and Mrs. Claudia Schogoleff for technical assistance.

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THE EFFECT OF HISTAMINE ANTAGONISTS ON GASTRIC SECRETION

J. E. BOURQUE AND E. R. LOEW[1]

Department of Physiology, Wayne University, College of Medicine, Detroit, Michigan

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Experimental studies concerning the effect of the histamine antagonist, thymoxyethyldiethylamine (929F), on gastric secretion induced by histamine (1) have been extended to determine the effect of 929F on gastric secretion induced by pilocarpine. In addition, the histamine antagonist (2, 3), N-phenyl-N-ethyl, N′-diethyl, ethylenediamine (1571F), was employed in experiments designed to ascertain whether the gastric secretory response to histamine could be suppressed or wholly eliminated. Pilocarpine and food were also used as secretory stimulants in an attempt to determine whether 1571F[2] acted specifically as a histamine antagonist. Any drug having the ability to specifically antagonize the powerful stimulating action of histamine on the gastric secretory glands could be advantageously used to investigate the relationship, if any, of histamine to gastric secretion following the ingestion of food or under other conditions.

Methods. Heidenhain pouch dogs trained to stand quietly in stocks were used. The animals were fasted for at least 20 hours before the experiments were begun.

All drugs were freshly prepared (5.0 per cent solution) and injected subcutaneously. 929F and 1571F were given in doses of 10 mgm./kgm. In the studies with 1571F and histamine, the double histamine test was employed as previously described (1). The maximal non-toxic dose for our dogs, 10 mgm./kgm., was injected 30 minutes before the second histamine injection.

Rectal temperatures were determined at 20 or 30 minute intervals before and for 3 hours following the administration of the drugs (929F or 1571F). This was considered necessary because in preliminary experiments we had found that 1571F in subcutaneous doses of 20 or 40 mgm./kgm. produced muscular tremors, convulsions and pyrexia. However, no significant temperature rises were noted in dogs treated with 10 mgm./kgm. of 1571F or 929F.

In the pilocarpine experiments a dose of 2.0 mgm. of pilocarpine hydrochloride was injected 30 minutes after 929F or 1571F. This dosage of pilocarpine was selected because we found that it induced a volume of gastric juice approximately equal to that obtained following the injection of 0.5 mgm. of histamine diphosphate, the dosage employed in the histamine experiments.

In the food experiments the stimulus consisted of a meal of 200 cc. of milk and 200 grams of Kennel Ration. Since the gastric secretory response to this

[1] Present address: Division Pharmacological Research, Parke, Davis & Co., Detroit, Michigan.

[2] The drugs 929F and 1571F were supplied by Parke, Davis & Co.

stimulus continues for 1 to 6 hours, a dose of 10 mgm./kgm. of 1571F was given one and three hours after the meal stimulus. Corresponding experiments with 929F were not conducted because it had been shown by Loew and Chickering (1), that this drug did not inhibit the histamine-induced gastric secretory response.

The gastric juice was collected and the volume recorded at 20 to 30 minute intervals. Each sample was then titrated *in toto* for free and total acid, using Töpfer's reagent and phenolphthalein, respectively, as indicators.

RESULTS. A. *1571F and double histamine.* In 15 experiments on 3 dogs injections of 1571F 30 minutes before the second histamine stimulus reduced the volume, total acidity and free acidity of the gastric juice, 21.9 per cent, 35.2 per cent, and 47.2 per cent respectively (table 1). These percentages were obtained by comparing the output after 1571F and the second histamine stimulus with control data consisting of the output after the first histamine injection.

TABLE 1

Gastric secretion following histamine stimulation in dogs pretreated with 1571F

	NUMBER OF EXPERIMENTS	NUMBER OF DOGS	VOLUME	TOTAL ACID	FREE ACID
			cc.	*mgm. HCl*	*mgm. HCl*
Control					
Histamine	15	3	15.5	52.2	46.4
Treated					
1571F and histamine	15	3	12.1	33.8	24.5
Variation from control (%)			−21.9	−35.2	−47.2
Control					
Histamine #1	15	3	16.4	52.9	46.9
Control					
Histamine #2	15	3	15.5	51.0	44.9
Variation of histamine #2 from histamine #1			−5.4	−3.5	−4.2

When pretreatment with 1571F was omitted the outputs of gastric juice following the first and second histamine injections were practically the same in volume, free acidity and total acidity (table 1). We have also noted that in a given dog under nearly identical conditions, the secretory output was fairly constant following the first and second histamine injections on the same and different days.

B. *1571F and pilocarpine.* In 15 experiments on 3 dogs the administration of 1571F 30 minutes before pilocarpine stimulation reduced the volume, total acidity and free acidity of the gastric juice, 13.5 per cent, 36.8 per cent and 47.2 per cent, respectively (table 2). This degree of inhibition is nearly identical to that which obtained when histamine was used in animals pretreated with 1571F.

C. *1571F and food.* In 10 experiments on 4 dogs administration of 1571F at one and three hours after the meal stimulus failed to modify the gastric secretion as compared with control experiments with food alone. The differ-

ence in mean values for volume, total acidity and free acidity did not exceed 5.0 per cent (table 2).

D. *929F and pilocarpine.* In 12 experiments on 3 dogs injections of 929F 30 minutes before pilocarpine failed significantly to depress the secretory output. The variations from control experiments were 12.0 per cent, 3.8 per cent, and 14.5 per cent for volume, total acidity and free acidity respectively (table 2).

Discussion. The degree of inhibition of gastric secretion produced by injections of 1571F in dogs stimulated with histamine or pilocarpine was nearly identical. In each instance, computation of the probable error of the mean differences ($M_1 - M_2 \pm$ P.E.) revealed that the data were statistically significant. No unequivocal evidence has been presented to indicate that pilocarpine exerts its stimulating effect on gastric secretion through the mediation

TABLE 2

Gastric secretion following pilocarpine stimulation or a meal stimulus in dogs treated with 929F or 1571F

	NUMBER OF EXPERIMENTS	NUMBER OF DOGS	VOLUME	TOTAL ACID	FREE ACID
			cc.	*mgm. HCl*	*mgm. HCl*
Control					
Pilocarpine	15	3	14.0	32.0	26.9
Treated					
1571F and pilocarpine	15	3	12.1	20.2	14.2
Variation from control (%)			−13.5	−36.8	−47.2
Control					
Pilocarpine	12	3	12.0	36.1	30.3
Treated					
929F and pilocarpine	12	3	13.4	34.7	25.9
Variation from control (%)			+12.0	−3.8	−14.5
Control					
Meal	10	4	91.8	419.8	387.1
Treated					
Meal and 2 doses 1571F	10	4	89.6	399.0	378.1
Variation from control (%)			−2.3	−4.9	−2.3

of histamine. Therefore our data do not suggest that 1571F specifically antagonizes the stimulating effect of histamine on the gastric secretory cells. It is possible, however, that 1571F antagonized the pharmacological activity referable to distinct chemical configurations present in both histamine and pilocarpine.

Even though 1571F reduced the secretory output following histamine and pilocarpine injections, it failed to inhibit the secretion induced by a meal stimulus. Hence it may be concluded that 1571F did not influence the humoral or hormonal factors related to gastric secretion which operate following a natural stimulus such as the intake of food.

Some investigators have presented evidence which suggests that the hormone, gastrin, may be identical with histamine. However, in relation to this question

it is interesting to note that in our experiments 1571F antagonized injected histamine but failed to antagonize the humoral and hormonal factors which elicit gastric secretion following a meal stimulus.

The inhibition of gastric secretion by 1571F in animals stimulated with either histamine or pilocarpine can not be referable to concomitant pyrexia. At no time during the periods following the administration of 1571F did the average rectal temperatures exceed by more than 0.3°F. the average control temperatures taken just before treatment with 1571F or during the histamine and pilocarpine control experiments. No depression of gastric secretion occurred in the food experiments where two doses of 1571F would be expected to increase pyrexic effects.

Since 929F potentiated the stimulating action of histamine on the gastric secretory cells (1), it was interesting to note that the drug failed to potentiate the action of pilocarpine.

Burchell and Varco (5) have recently reported their inability to demonstrate that the two histamine antagonists, 929F and 1571F, diminish the secretory response of gastric pouches (Heidenhain) following histamine stimulation. The dose of histamine which they employed was several times greater than the minimal amount which we found adequate to induce a moderate gastric secretory response in our animals. Our control data and the data reported by others (6) firmly establish the reliability of the double-histamine assay procedure when proper precautions are taken regarding the number of experiments conducted, the health of the animals, and the use of non-toxic doses of drugs or biological preparations. Hyperpyrexia referable to parenteral administration of substances which contain pyrogens, or to muscular tremors or convulsions coincident with drug action will reduce gastric secretion.

It was not possible to employ larger doses of 1571F or 929F in an attempt to determine whether these drugs antagonize the effect of histamine on the gastric cells. The former drug, in doses of 20 and 40 mgm./kgm., subcutaneously, caused normal dogs to become apprehensive, nervous, excitable and, finally, produced muscular tremors, tonic convulsions, and hyperpyrexia. The reactions were noted 15 to 45 minutes after administration of the drug. It was previously mentioned (1) that 929F induces retching, vomiting, and defecation when quantities of 20 or 30 mgm./kgm. were administered subcutaneously. Others (2–5) have commented on the symptoms and untoward reactions observed following administration of 929F and 1571F.

CONCLUSIONS

1. The data do not support the belief that 1571F is a specific antagonist to histamine. Although the drug reduced the gastric secretion in dogs with gastric pouches (Heidenhain) following histamine stimulation, the reduction in secretion was nearly identical after pilocarpine stimulation. 1571F did not modify the gastric secretion of gastric pouch dogs stimulated with food.

2. Pretreatment with 929F failed significantly to modify the secretion from gastric pouches in dogs injected with pilocarpine.

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THE DEVELOPMENT OF RESISTANCE BY RATS AND GUINEA PIGS TO AMOUNTS OF TRAUMA USUALLY FATAL[1]

R. L. NOBLE

From the Research Institute of Endocrinology, McGill University, Montreal

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A method for the experimental production of graded amounts of trauma has been previously studied in this laboratory and described by Noble and Collip (1). It was found that animals which died following trauma showed many of the characteristic findings ascribed to the condition of "shock". The observations presented supported the hypothesis that the production of a toxic substance in the tissues was the etiological factor in traumatic shock. The present paper reports a continuation of research based on the toxin theory of shock. Rats and guinea pigs have been subjected to trauma by the previously described method. Since this procedure did not require the animal to be anesthetized and caused only a minimal degree of hemorrhage, it was especially suited for a study of the effects of repeated trauma. In the initial experiments it was found that rats subjected to small amounts of trauma rapidly acquired a resistance so that they could withstand a degree of trauma otherwise fatal. A detailed study of this phenomenon forms the basis for this communication.

METHODS. Since the procedure used to produce trauma has been previously described in detail (1), only a brief résumé is included. The animal, after taping of the paws, is placed in a circular drum which has two projections on the side. During rotation of the drum at a speed of 42 r.p.m. the animal is alternately carried a short distance up the side on the projection and then dropped. The amount of trauma, therefore, is directly related to the number of turns of the drum, and is reflected on the mortality curve. The animals used in these experiments have been maintained under the same conditions as those previously described, so that the results would be comparable.

To make rats resistant it has been customary to use an animal of 120 to 140 grams and subject it initially to 200–300 turns, since this seldom causes mortality. This is repeated after 2 days, and at intervals of 2 or 3 days the number of turns is increased to 400, 600 and 800. Over a period of from 12 to 14 days the animals are raised from 200 to 1000 turns. As a standard procedure they are subjected to 1000 turns every week thereafter until used for other experiments. With guinea pigs the same principle is followed, except that due to their increased susceptibility only 125 turns are given initially, and the number is increased by 25 to 50 over a slightly longer period than for rats. The speed with which the number of turns may be increased can be judged by following the weight curves of the animals. With correct spacing the animals do not lose in weight after the initial run. For adrenalectomized animals the number of initial

[1] The findings recorded in this paper are a continuation of those contained in reports submitted to the National Research Council of Canada, dating from 30th July, 1941.

turns is reduced, and then raised more slowly, due to their increased susceptibility.

RESULTS. *Production of resistance in rats.* When an untreated rat is subjected to 1000 turns initially it has been found in a large series of experiments that only very exceptionally does an animal survive. This appears to be true in rats weighing from 120 to 300 grams. Although large rats are slightly more resistant than small ones, the amount of trauma following 1000 turns is of sufficient magnitude to cause 98 to 100 per cent mortality. Rats which are able to tolerate 1000 turns successfully are referred to for the purposes of this paper as resistant animals. At present, out of 106 male rats 105 have been made resistant, and have withstood 1000 turns on at least 3 occasions. During the development of resistance only one rat has died, and that was following the initial run. At present, therefore, 105 animals have been subjected to an amount of trauma almost invariably fatal to an untreated rat, without mortality. Actually these 105 rats have received 1000 turns a total of 919 times. In these experiments the amount of trauma was increased gradually, as indicated. In most cases the time intervals and amount of trauma were judged by the weight curves of the animals. After the initial run some loss in weight usually occurred over 24 hours. Following this, however, the rats gained weight slowly despite subsequent trauma. If a loss in weight continued or was marked, the number of turns was lowered and a longer rest period given between runs. In general, however, the animals appeared to develop a resistance with unexpected rapidity. Once the rats were made resistant they have received 1000 turns weekly or twice weekly. In most cases this has continued for 5 or 6 weeks before the animals were used for other experiments. In one case 8 rats have received 1000 turns on 26 occasions over the past $6\frac{1}{2}$ months, and at present show no indication of ill health or loss of resistance.

Appearance of resistant rats. Following the first few runs the rats appeared much less affected by the trauma than would be expected from observations on control animals. When a resistant rat was subjected to 1000 turns its general appearance differed markedly from that of an animal subjected initially to severe trauma. A few moments after removal from the drum the resistant rat appears quite normal, runs about the cage, exhibits a normal response to outside stimuli, and never shows the prostration or collapse described as typical of this form of trauma. Such animals do not drink excessively and there is no initial phase of anuria; in some cases mild polyuria may be found. Subsequently, they do not develop diarrhea.

If a resistant rat is examined after receiving 1000 turns the appearance is in sharp contrast to an animal killed by trauma. The heart appears to continue to beat strongly and is not congested or dilated; the cut tissues bleed readily—suggesting a normal blood pressure; very little bruising or hemorrhage can be found even over the bony prominences. The abdominal organs and gastro-intestinal tract appear essentially normal and show no sign of the congested condition seen in unresistant rats. The lungs and kidneys are not affected. Congestion of the brain is absent and sub-arachnoid hemorrhage is not seen.

The adrenals appear to be within normal limits. If an animal which has only recently been made resistant to 1000 turns is killed, it will not, however, show such a normal picture as outlined above. In this case some of the changes previously reported for normal rats may be found, although they are of much less intensity. As the degree of resistance of the animal increases the abnormal findings become progressively less.

Blood findings in resistant rats. As the general appearance of the resistant animal after trauma would suggest, little evidence of concentration of the blood has been found. The usual hemoconcentration associated with this form of trauma in the normal rat was not encountered in the resistant animal. Changes in hemoglobin values have been very small when followed for individual rats and have indicated that, if anything, hemodilution to a mild degree may follow trauma. In addition, it has been noted that the hemoglobin values of resistant rats tend, as an average, to be slightly lower than those of normal rats, although marked variations occur.

It has been noted that normal rats which die immediately following trauma develop rigor mortis after a few minutes. However, when resistant rats were killed following trauma this immediate rigor mortis did not take place (although it sets in later, as is found when a control untraumatized rat is killed). Lactic acid determinations on the blood and muscle of some of these animals were kindly carried out by Dr. A.H. Neufeld, of this Institute. In the normal rat after trauma very high figures were found; the blood increased from a normal of 20 to 30 mgm. per 100 cc. to 100 to 200 mgm. or even higher. In the case of 7 resistant rats subjected to 1000 turns the blood lactate was much lower and ranged from 26 to 53, an average of 40.4 mgm. per 100 cc. Other studies have failed to demonstrate that the resistant rat can handle lactic acid more readily than the normal.

Factors affecting resistance. Although for the purposes of this paper rats are considered resistant when they survive 1000 turns, it is possible to continue to increase this considerably. In one case 4 rats were rapidly raised to 1000 turns in 12 days. Then at 5 to 6 day intervals the number was increased to 1500, 1800, 2000, 2500 and 3000. One rat died after 1500, two after 2500, and one after 3000. It is believed that if the increase in trauma had been made more gradual, that even greater resistance would have developed. After such prolonged periods of trauma some of the rats may have difficulty in eating, due to broken teeth, etc., and special care is required to maintain their general condition.

The amount of trauma necessary for the development of detectable resistance has been investigated by a different type of experimental procedure. In this case 12 rats received 300 turns on two successive days. At 3, 8 and 15 days after the initial run each group of 4 received 1000 turns. In the first two cases only evidence that some resistance had developed was indicated by a lowered percentage mortality, or as an increased survival time before death (2 to 48 hrs.). This form of experiment would appear to be advantageous where a long survival time is desired, since with normal animals death occurs so rapidly after severe trauma that they are unsatisfactory for certain types of tests.

A number of rats have been set aside to determine the length of time the resistance continues after the last exposure to trauma. The rats were made resistant in the usual way and received 1000 revolutions on at least 3 occasions. After an interval of one month 9 of 10 animals retained their resistance to 1000 turns. After 3, 4 and 5 months 2 of 3 rats survived in each case. The resistance, when once established, therefore, persisted for a considerable period of time. During an epidemic of gastro-enteritis which developed in the rat colony it was noted that 4 resistant rats had apparently contracted the disease. While still in relatively good health, the animals received 1000 turns, and in each case death followed.

Resistance after anesthesia. Despite the fact that the legs of the animals were taped together, it was suggested that the resistance might be related to the rat learning how to fall and minimize the degree of trauma. To rule out this factor, 3 resistant rats were anesthetized with nembutal and then given 1000 turns. All these animals survived, although co-ordinate movements were abolished and muscular relaxation was complete. There was no evidence of rupture of the spleen, as frequently occurred in normal rats traumatized while under nembutal anesthesia.

Resistance in adrenalectomised rats. Two sets of experiments were devised to determine whether adrenalectomised rats could be made resistant. In the first group the rats were maintained by drinking 0.9 per cent NaCl instead of water, and the second group in addition received pellets (80–90 mgm.) of desoxycorticosterone acetate implanted subcutaneously. Animals treated in such a manner are, as previously reported, very sensitive to trauma, so that after 500 turns a mortality of 75 to 85 per cent is found. In order to make these animals resistant, they received 200 turns initially, and the amount of trauma was increased by 100 turns over 2 to 3 weeks. Of the first group of 8 rats, three died early in the experiment after 400 turns, five tolerated 600 turns successfully, and one rat survived after 900. This rat was then given water to drink instead of saline and died in 4 days. In the second group of 6 rats all tolerated 600 turns, and four 700 turns.

Resistance in guinea pigs. It has been found that guinea pigs readily may be made resistant by a method similar to that described for rats. Since this species shows almost 100 per cent mortality after 300 turns, it is necessary to commence with only 125 turns. After 4 to 5 day intervals the number of turns was increased 25 at a time. Starting with 16 animals, it was possible to have 13 of them survive after 400, and 3 animals were taken as high as 600 turns. During this process some animals were subjected to trauma as often as 26 times.

Discussion. The results which have been obtained show that by gradually increasing the amount of trauma, rats and guinea pigs eventually become resistant to normally fatal doses. Since it is believed that the drum method of applying trauma results in a condition of shock, it would appear that animals may be made resistant to this condition. The normal and the resistant animal show a striking difference in appearance after exposure to trauma. Whereas the normal animal is obviously affected and dies with severe diverse pathological

changes, the resistant rat appears normal in every respect. Concentration of the blood does not occur in the resistant rat. It has been possible to expose 165 rats, without mortality, to degrees of trauma almost inevitably fatal to normal rats. The type of resistance which develops appears to have some curious features. The onset is rapid, and in a very short time the animal can withstand large amounts of trauma. Once the resistant state is established it has not shown any tendency to diminish even though rats have received trauma over periods of 6½ months. However, 4 rats which contracted gastro-enteritis apparently lost their resistance to trauma. The resistant condition may persist for several months after the last exposure to trauma. Since the actual mechanism of death in the animals exposed to this type of trauma has not yet been clearly defined, it is difficult to postulate what type of resistance develops. Such factors as "learning" have been ruled out by the demonstration that even when animals were completely anesthetised they maintained their resistance. Since the preliminary research on this type of trauma was instituted, the view was held that the results could best be explained by the theory of a liberation of a toxic substance by damaged tissue. It is possible, if this theory is correct, that the resistant animals become immunized by the production of some antitoxin. In this connection experiments designed to transfer protection by the blood of resistant animals have so far proved unsuccessful. However, at present it is thought that some such detoxifying mechanism is involved and that further work may clarify the question of whether active or passive immunization may be possible. Whether rats resistant to trauma inflicted by the drum method are resistant to other forms of trauma has not yet been established.

Using the drum method for producing trauma it has been difficult to assess to what degree direct mechanical damage might be related to the death of the animal. Some concussion of the brain and nervous system must result from the nature of the trauma, and it has been suggested that intracranial hemorrhage may be a factor in producing mortality. Whether animals showing evidence of brain hemorrhage should be discarded from the results is difficult to assess. In the case of rats in the present work, this has not been considered as a separate factor. When resistant rats are subjected to even extreme degrees of trauma, they do not die, and postmortem examination has not revealed any brain hemorrhage. In this case one would expect any type of mechanical injury to be more prominent than in the case of normal animals. It is possible, however, that with the resistant state the results of mechanical damage are minimized in some manner. In a previous paper (2) it was reported that treatment with adrenal cortical extracts, while restoring the resistance of adrenalectomized rats, would only slightly raise the resistance of normal rats to trauma. The observations in this paper would indicate that the adrenal glands are not essential for the development of resistance to trauma. In the case of adrenalectomized rats, as in normals, it has been possible to make them resistant to usually fatal degrees of trauma. Whereas the adrenals undoubtedly play some rôle in the general maintenance of the animal's normal tolerance to trauma, they are not essential for the development of the special type of resistance described.

Up to the present time the procedure described is the only treatment after which rats have consistently survived 1000 turns. In a recent report (3) it was found that ascorbic acid prevented mortality following a certain type of trauma. Using the drum method as described, pretreatment with ascorbic acid in doses of 300 mgm. per kgm., either by single or repeated subcutaneous injection, or by mouth, has not prevented mortality after 1000 turns.

From the findings reported it has been possible to establish a colony of resistant rats and use them for comparative studies with normal and traumatized rats. It is hoped that with such animals for assay purposes the investigations for a specific toxic substance in tissues may be expedited.

SUMMARY

By gradually increasing the degree of trauma, as applied by the drum method, it has been possible to make rats and guinea pigs resistant to amounts of trauma fatal to normal animals.

Resistant animals, when exposed to severe trauma, appear normal in every respect and do not show any of the signs of shock as encountered in normal animals.

The resistant condition persists after numerous repetitions of trauma, is not affected by nembutal anesthesia, and is still present some months after the last exposure to trauma.

Adrenalectomized rats have been found to develop a resistant state to trauma similar to intact animals.

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EFFECT OF THE TOTAL LOSS OF PANCREATIC JUICE ON THE BLOOD AND LIVER LIPIDS[1]

J. GARROTT ALLEN, C. VERMEULEN, FREDERICK M. OWENS, JR. AND LESTER R. DRAGSTEDT

From the Department of Surgery, University of Chicago

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The status of lipocaic as an internal secretion of the pancreas depends in part upon recognition of the fact that the depancreatized animal is not returned to a normal state by the adequate administration of insulin. There is nearly general agreement at the present time that the depancreatized dog will not survive for long if treated only with insulin and that at death a profound infiltration of fat in the liver is usually found. Animals maintained on high fat diets may develop severe fatty livers and die within six weeks, whereas those fed a low fat or fat-free diet may survive for many months (1). The oral administration of fresh pancreas corrects the deficiency and when given along with insulin permits the depancreatized dog to survive indefinitely in a good nutritive state. The conclusion from this laboratory (2) that the beneficial effect of pancreas in this connection cannot be accounted for on the basis of its content of lecithin or choline has been accepted by Best (3) and McHenry (4), both of whom have contributed so much to our understanding of the lipotrophic action of these substances. That pancreatic enzymes are not necessary for this effect is indicated by the fact that pure choline is active and also by the fact that preparations of lipocaic free from pancreatic enzymes have been secured (1). There remains the possibility that the specific substance in pancreas, lipocaic, is excreted in the pancreatic juice and normally reabsorbed from the intestine. In this event the total deviation of the pancreatic secretion to the exterior by means of an appropriate fistula might be expected to cause fatty infiltration of the liver and the other sequelae of lipocaic deprivation.

METHODS. The total external loss of pancreatic juice was accomplished by a minor modification of the method previously described from this laboratory (5). Adult female dogs were operated upon under morphine-ether anesthesia and with the usual aseptic precautions. The pancreas was separated from the first portion of the duodenum to a point 2 cm. below the entrance of the common bile duct. Several small pancreatic ducts were usually divided and ligated in this process. The duodenum was then transected immediately below the bile duct and again 6 cm. lower down. The short segment of duodenum thus isolated contained the lower major pancreatic duct. A gold plated cannula was placed in this isolated segment, the ends closed, and the whole carefully wrapped in

[1] This work has been aided by grants from the Josiah Macy Jr. Foundation, the Committee on Research in Endocrinology of the National Research Council, the Eli Lilly Company, and the Douglas Smith Foundation for Medical Research of the University of Chicago.

omentum. The cannula was led to the outside through a small stab wound. The remaining proximal and distal ends of the duodenum were anastomosed by end to end suture. The juice was collected continuously throughout the life of the animal by means of sealed rubber bags attached to the cannula. The amount secreted ranged from 500 to 2000 cc. daily and necessitated the intravenous administration of large amounts of Ringer's solution to prevent acidosis and dehydration. Calcium carbonate and powdered bone meal were given by

TABLE 1

The effect of complete loss of pancreatic juice on the blood lipids

DOG NO.	WEEKS AFTER PRODUCTION OF PANCREATIC FISTULA	TOTAL BLOOD LIPIDS	DOG. NO.	WEEKS AFTER PRODUCTION OF PANCREATIC FISTULA	TOTAL BLOOD LIPIDS
		mgm. per cent			*mgm. per cent*
11	Control	710	16	Control	640
	2	742		2	587
	3	601		3	727
	4	760		4	620
	5	596		5	533
12	Control	608		6	540
	2	722		7	607
	3	928		8	505
	4	917	17	Control	644
13	Control	670		1	663
	1	640		2	550
	3	688		3	773
	4	680		4	580
	5	660		5	833
14	Control	457		6	613
	2	560		7	777
	4	602		8	540
15	Control	666		9	533
	4	629		10	410
				11	465
				12	297
				13	370
				14	537
				16	587
				17	513
				18	702
				19	670

mouth to prevent the development of gastric and duodenal ulcers. The diet was that commonly given to depancreatized dogs in this laboratory and consisted of white bread, whole milk, meat and suet, supplemented with cod liver oil and brewer's yeast. The fat content of this diet is approximately 40 per cent.

The blood lipids were determined by a modification of Bloor's method on weekly samples taken from certain of the animals as indicated in table 1. An autopsy was performed at the conclusion of the experiment to determine the

condition of the liver and pancreas and to make certain that no ducts remained whereby pancreatic juice could enter the duodenum. The amount of fat in the liver was estimated by gross and microscopic examination and in nine of the animals also by chemical analysis using the method previously described (1).

RESULTS. The data are summarized in tables 1 and 2. They show that deviation of the pancreatic juice from the intestinal tract by means of an external fistula does not produce the fatty infiltration of the liver and the hypolipemia so commonly seen in the insulin treated depancreatized dog.

Seventeen animals survived the complete loss of pancreatic juice for periods ranging from 4 to 50 weeks. The loss of sodium was compensated for by the

TABLE 2

The effect of complete loss of pancreatic juice on liver fat

DOG. NO.	WEEKS AFTER PRODUCTION OF PANCREATIC FISTULA	LIVER FAT PER CENT WET WT.	GROSS AND MICROSCOPIC APPEARANCE OF LIVER	REMARKS
1	12		Normal	Died following biopsy of liver
2	7		Normal	Sacrificed
3	6		Normal	Sacrificed
4	50		Normal	Sacrificed
5	4		Normal	Sacrificed, cannula withdrawn
6	6		Normal	Sacrificed, cannula withdrawn
7	8		Normal	Sacrificed
8	9		Normal	Sacrificed
9	5	6.0	Normal	Sacrificed, cannula withdrawn
10	4	4.0	Normal	Sacrificed
11	6	5.0	Normal	Perforated gastric ulcer
12	4	5.8	Normal	Abscess of thigh, sacrificed
13	6	5.8	Normal	Cannula withdrawn, sacrificed
14	6	6.2	Normal	Cannula withdrawn, sacrificed
15	4	12.1	Slight fat infiltration	Large subphrenic abscess, sacrificed
16	8	8.2	Normal	Cannula withdrawn, sacrificed
17	20	14.7	Slight fat infiltration	Cannula withdrawn, sacrificed

daily intravenous injection of Ringer's solution and the oral administration of alkalies. Death resulted usually from withdrawal of the cannula, infection or perforated ulcer. In every case the pancreas appeared normal, free from infection, and no ducts leading to the duodenum were found. In 14 of the animals the amount of fat in the liver was within the normal range; in 3 an increased amount of fat was found.

Repeated estimations of the serum lipids were made in seven of the fistula animals and recorded in table 1. In general they remained within the normal range. In one case (17) a low value was found on two occasions after 3 months, but the lipids returned to a normal level and remained so for a month before the animal died.

DISCUSSION. The failure of total deviation of pancreatic juice from the

intestine in these experiments to reproduce the fatty infiltration of the liver so commonly seen in insulin treated pancreatectomized dogs indicates that this abnormality is not due to the failure of the pancreatic enzymes or of other substances in the pancreatic secretion to reach the intestine. This finding supports the conclusion of Prohaska, Dragstedt and Harms (2), derived from a similar type of experiment. In this report seven dogs with complete external pancreatic fistulae were described with survival periods of 30, 36, 23, 26, 35, 39 and 43 days. Fatty liver was absent in four of these animals and present to a slight degree in three. Of the latter three, two were found to have extensive subcutaneous abscesses and one died of peritonitis. We have noted on many occasions the association of fatty infiltration of the liver with infections. In the present series one of the animals (15) in which increased liver fat was found had a large subphrenic abscess. If the four cases complicated by extensive infection are excluded there remains a total of 19 animals with complete pancreatic fistulae in the two series with only two in which an increased amount of liver fat (14.7 and 8.2 per cent respectively) developed. These animals survived the loss of pancreatic juice for an average of $8\frac{1}{2}$ weeks.

These findings become more significant when compared with the incidence of fatty infiltration of the liver in depancreatized dogs maintained on the same diet. Thus, of 25 such animals, in a consecutive series, treated with regular insulin, 8 developed a fatty liver in excess of 34 per cent within 30 days, 11 a fatty liver in excess of 25 per cent within 35 days, and the remaining 6 a fatty liver in excess of 14 per cent within 30 days.

The failure of the animals with total pancreatic fistulae to display the marked hypo-lipemia, so commonly seen in depancreatized dogs with the fatty livers of lipocaic deficiency, indicates that this abnormality is also not due to the absence of pancreatic juice from the intestine. This finding is interesting in view of the fact that these animals develop the same bulky stools and steatorrhea seen following complete pancreatectomy. Apparently the decrease in blood fat in the latter case is not due to the impaired absorption from the intestines.

A recent paper by Montgomery, Entenman, Chaikoff and Nelson (6) should be discussed in connection with these experiments. These workers removed the pancreas from eight dogs. The animals were all given a diet low in fat as compared to the one used in our work. Three of the dogs received no supplemental treatment, were sacrificed after 20 weeks, and were found to have fatty livers with a total fatty acid content of 15.1 to 20.5 per cent. Three dogs were fed 250 grams of raw pancreas and 377 cc. of pancreatic juice daily for 8 weeks and thereafter the same amount of pancreatic juice but no pancreas for 20 weeks. At the close of this period the liver fatty acids were found to be 3.7, 7.0 and 5.79 per cent respectively. The remaining two animals were given 250 grams of raw pancreas and pancreatic juice daily for 4 and 3 weeks respectively and then only pancreatic juice for 20 weeks. At the close of this period the liver fatty acids were 3.11 and 3.19 per cent. As a result of this experiment the authors concluded that the fatty livers produced by excision of the pancreas can be completely prevented by the daily ingestion of fresh pancreatic juice.

We believe this conclusion is not warranted for several reasons. In the first place the control is not adequate. The experimental animals were fed large amounts of raw pancreas (250 grams daily) for periods of from 3 to 8 weeks in addition to the pancreatic juice whereas the control animals were given no preliminary treatment with raw pancreas. Secondly, we believe it is necessary to adopt much more rigid criteria before concluding that any given substance, i.e., pancreatic juice or pancreas extract, contains lipocaic. Those employed in this laboratory are described by Dragstedt, Vermeulen, Goodpasture, Donovan and Geer (7). In the present instance a minimum requirement would evidently be to demonstrate that the oral administration of pancreatic juice can relieve a definitely established syndrome of lipocaic deficiency in depancreatized dogs, i.e., cure the fatty liver as checked by biopsy, restore the abnormal glucose excretion and insulin tolerance, relieve the hypolipemia and improve the nutritive state. This is necessary in view of the results with total pancreatic fistula presented in this paper and the finding of Prohaska, Dragstedt and Harms (2) that the oral administration of as much as 1000 cc. of activated pancreatic juice per day could not prevent the development of extreme fatty liver in depancreatized dogs. It seems unlikely that lipocaic present in pancreatic juice would be destroyed in the activated juice since the oral administration of pancreas or pancreas extracts is effective. This fact demonstrates that the active principle in pancreas is not readily destroyed by the digestive enzymes.

CONCLUSIONS

1. The total loss of pancreatic juice from the body by complete pancreatic fistula does not produce the hypolipemia or fatty infiltration of the liver so commonly seen in the insulin treated depancreatized dog.

2. The conclusion that lipocaic is not present in pancreatic juice to any significant extent is confirmed.

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STUDIES ON THE GLYCOGEN METABOLISM OF ATROPHIC AND REGENERATING MUSCLE[1]

B. LAZERE, J. D. THOMSON AND H. M. HINES

From the Department of Physiology, State University of Iowa

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Changes in the concentration of numerous chemical constituents in skeletal muscle undergoing atrophy as a result of denervation can largely be accounted for by progressive changes in the relative amounts of muscle cell and connective tissue phases. Such changes are necessarily limited to those constituents exhibiting an unequal phase distribution in normal muscle. Muscle glycogen presents an important exception to this interrelationship between muscle phases and chemical concentration (1). Following denervation, muscle glycogen undergoes a decrease in concentration at a velocity which markedly exceeds the rate of concomitant phase changes. Moreover, the glycogen of atrophic muscle is not increased by glucose ingestion and is more susceptible than that of normal muscle to the glycogenolytic effects of exogenous adrenalin, insulin and thyroxin (2).

This report is concerned with a search for possible causes of the altered glycogen metabolism exhibited by denervated muscle. The changes in glycogen concentration have been correlated with other chemical and functional changes occurring during the course of atrophy and subsequent regeneration. A comparative study has been made of the glycogen changes in muscles undergoing atrophy from causes other than denervation.

METHODS. These studies were carried out on the gastrocnemius muscle of adult albino rats. The general procedure was a concurrent analysis of the corresponding muscles of the two hind limbs, one muscle serving as the control for its contralateral experimental member. Unless otherwise indicated, the data for the atrophic muscles are expressed in percent of their contralateral controls.

Glycogen, creatine, fibrillation, tension and weight measurements were carried out at suitable intervals during the course of atrophy and regeneration following cast application, denervation and tenotomy. Complete denervation of the gastrocnemius was accomplished by crushing the tibial nerve at the level of its junction with the peroneal (3). A total of 215 animals was employed in the denervation studies. The muscles of 74 animals were subjected to tenotomy by clipping off the os calcis, freeing the tendon from all body attachments and sewing the cut end of the tendon to the skin. In 26 animals one hind limb was immobilized by encasement in plaster bandages from the toes to the hip joint. The cast was allowed to harden with the foot in the position of mid-extension. To allow regenerative processes following immobilization, the casts were removed from some animals after being worn for a period of 10 days. Care was taken to

[1] Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

reject the few animals which showed evidence of circulatory impairment due to ill-fitting casts.

Measurements of muscle creatine, tension and mass were necessarily carried out on animals different from those employed for the glycogen determinations. The methods employed for measuring muscle tension and stimulating muscle and nerve have been described elsewhere (3). The techniques employed for muscle removal, glycogen precipitation and hydrolysis were essentially those described in previous studies (2). The animals chosen for the different experiments were paired as to age, sex and nutritional status.

Fibrillary activity was detected by direct observation of the exposed muscle in reflected light and also by amplification of action potentials led from the muscle through two needle electrodes and visualized on the screen of a cathode ray oscillograph.

Six animals were subjected to bilateral adrenalectomy and section of the left sciatic nerve at a single operation. These animals then received daily subcutaneous injections of cortical extract (Upjohn) in amounts of 13 rat units per kilogram of body weight. The muscles were removed for glycogen determinations 7 days after the operation.

A study was made of the effects of quinidine administration upon fibrillary activity and muscle glycogen in two groups of animals. To one group of 7 animals the quinidine was administered between 48 and 72 hours after denervation, the time during which the denervated muscle experiences the greatest fall in glycogen concentration. The dosage was 100 mgm. of quinidine sulfate per kilogram of body weight injected intraperitoneally every six hours. We have confirmed the observations of Solandt and Magladery (4) that quinidine in these doses abolished or greatly lessened the fibrillary activity of denervated muscle. In order to minimize glycogen changes in the control muscle due to convulsive states occasionally induced by quinidine, the nerve to the control muscle was sectioned 24 hours prior to the removal of the tissue for analysis. This was considered permissible inasmuch as previous studies (2) have shown that glycogen metabolism is essentially normal in muscle for this period of time after denervation. To another group of 5 animals a single dose of quinidine was administered, followed 2 hours later by 0.75 gram of glucose per 100 grams of body weight given through a stomach tube. The animals were sacrificed 3 hours after glucose administration and the muscles analyzed for glycogen.

The sciatic nerves to both limbs were sectioned in 5 animals. The denervated gastrocnemius of one limb was subjected to electrical stimulation under light ether anesthesia for a period of 3 minutes twice daily. The stimuli consisted of 60 strong induction shocks per second and were applied to the muscle through needle electrodes which pierced the skin. The non-stimulated denervated muscle of the contralateral limb served as a control. Muscles were removed for glycogen determinations 78 hours after denervation and 8 hours after the last period of stimulation. Similar experiments were carried out on 6 animals in which 70 hours were allowed for degeneration of the severed nerve before commencement of electrical stimulation. Muscles were removed for glycogen de-

terminations 126 hours after denervation and 8 hours after the last period of stimulation.

RESULTS. Denervation, immobilization by casts and tenotomy were followed in a few days by comparable marked falls in the glycogen concentration of the affected muscles (fig. 1). The decrease in glycogen concentration occurred at

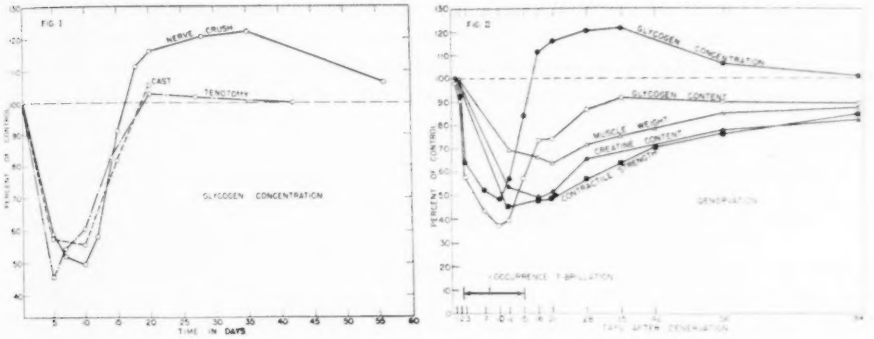

Fig. 1. A comparison of the changes in muscle glycogen concentration following cast application and removal, denervation and tenotomy.

Fig. 2. The relation of glycogen changes to other phenomena in skeletal muscle following denervation by nerve crush.

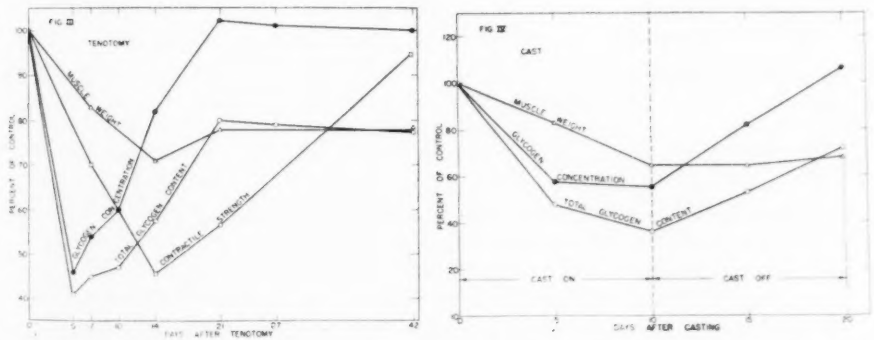

Fig. 3. Average values for the changes in muscle weight, strength and glycogen stores during atrophy and regeneration following tenotomy.

Fig. 4. Illustrating the rates of change in muscle weights and glycogen levels following immobilization by cast and subsequent cast removal.

a much greater velocity than the loss of muscle creatine, contractile strength or weight (figs. 2, 3 and 4). Conversely, restoration of muscle function through regeneration of the crushed nerve, regrowth of the tendon attachment and removal of the cast was associated with a rapid and complete restoration of glycogen concentration to normal levels (fig. 1). This occurred at a higher velocity

than the recovery of muscle mass, strength or creatine concentration (figs. 2, 3 and 4). At certain times during the course of regeneration, the muscles previously subjected to denervation exhibited glycogen concentration values considerably above those found in their contralateral controls. Significant increases above normal values were not observed in the muscles recovering from tenotomy.

Fibrillary activity first appeared on the second or third day after nerve crushing and disappeared on about the fifteenth day, a time shortly after the nerve had undergone sufficient regeneration to cause upon stimulation the development of measurable amounts of tension in its muscle. Fibrillary activity was not detected in either tenotomized or casted muscle.

TABLE 1

A summary of the effects of adrenalectomy, electrical stimulation and quinidine upon the glycogen concentration in control and denervated muscle

CONDITION OF ANIMAL	HOURS AFTER DENERVATION	GLYCOGEN CONCENTRATION*		GLYCOGEN CONCENTRATION AS PER CENT OF CONTRALATERAL CONTROL
		denervated	control	
Adrenalectomized	168	326	546	60
Control	168	350	670	52
Quinidine	72	240	422	57
Control	72	414	647	64
Quinidine + glucose	72	306	753	41
Control	72	378	740	51
Electrical stimulation	78	490		178
Control**	78	276		
Electrical stimulation	126	494		182
Control**	126	271		

* Milligrams per 100 grams. muscle expressed as glucose equivalent.

** Control was denervated non-stimulated contralateral muscle.

Administration of quinidine in doses sufficient to abolish fibrillary activity failed to prevent the usual fall in glycogen concentration following denervation (table 1). Moreover, the afibrillary quinidinized muscle did not experience an increase in its glycogen values following glucose ingestion. In this respect it behaved like denervated muscles in animals not subjected to quinidine administration. It should be pointed out that glucose ingestion resulted in an appreciable increase of glycogen storage in the control muscles of both quinidinized and and non-quinidinized animals. The animals subjected to adrenalectomy and sustained by adequate doses of adrenalin-free cortical extract experienced essentially the same drop in the glycogen concentration of their denervated muscles as did control non-adrenalectomized animals (table 1). Electrical stimulation of denervated muscle resulted in higher glycogen concentrations than existed in

the contralateral non-stimulated denervated control muscle. The stimulation was effective when initiated either immediately after denervation or after complete degeneration of the motor nerve (table 1).

Discussion. The glycogen concentration in skeletal muscle proved to be a sensitive indicator for the onset of changes characteristic of atrophy and regeneration. The decrease in glycogen concentration in denervated muscle exceeded in velocity the loss of creatine, mass or strength. Conversely, the onset of functional reinnervation was accompanied by a rapid and complete restoration of glycogen concentration which occurred sooner than the recovery of muscle mass, strength or creatine concentration.

An occurrence of essentially the same degree of glycogen loss from the denervated muscles of adrenalectomized animals as from the denervated muscles of control animals indicates that the cause for the disturbance in glycogen metabolism cannot be attributed to the glycogenolytic action of endogenous adrenalin on a tissue sensitized by denervation.

The time of onset for the rapid decline in glycogen concentration was concomitant with a loss of excitability in the peripheral portion of the crushed nerve and the appearance of fibrillary contractions in the denervated muscle. The subsequent recovery of glycogen concentration to normal or above normal values occurred at a time when the regenerating nerve upon stimulation was capable of eliciting a measurable amount of tension in its muscle. This time for the glycogen recovery also approximated the time for the disappearance of fibrillary activity from the muscle. Time relationships for the above changes strongly suggest that the depletion of glycogen may be due to the effects of fibrillary activity. Our experiments, however, offer three lines of evidence against such a cause and effect relationship. The finding that quinidine administration was able to abolish fibrillary activity without preventing the usual fall in glycogen concentration is considered to be evidence against the idea that the low glycogen concentration in denervated muscle is due to "overwork" or exhaustion from fibrillary activity. The finding that electrical stimulation of the paralyzed muscle resulted in higher glycogen levels rather than further decreases likewise argues against the "overwork" theory. Furthermore, the finding of equally rapid decreases in muscle glycogen levels following immobilization by casts and tenotomy, conditions in which fibrillary activity is absent, strongly suggests that the causes for the altered glycogen metabolism must be sought elsewhere.

The striking similarities in time relationships for the glycogen changes in three types of muscle atrophy and subsequent regeneration suggest that the causes for such may reside in some basic factor common to denervation, immobilization and tenotomy. It is suggested that this factor may be a decrease in metabolism associated with a reduced development of tension by the muscles. In each of the atrophic conditions there was a decrease in the ability of the muscle to produce tension and the metabolism associated therewith. Reflex tension was abolished by denervation and greatly lessened by the loss of the stretch stimuli to the proprioceptive receptors after immobilization and tenotomy. It appeared quite possible that the absence of normal muscle contractions resulted in insuffi-

cient energy levels for the coupled reactions that may be required for a normal rate of glycogen synthesis (5). The progressive resumption of normal types and degrees of activity following reinnervation and release from immobilization would provide the energy conditions favorable for glycogen synthesis at rates adequate for the establishment of pre-atrophy levels. It may be presumed that by electrical stimulation of paralyzed muscle the necessary energy for maintenance of a higher concentration of glycogen than existed in the non-stimulated denervated muscles becomes available. It is not improbable that energy level factors may be involved in the loss of protein from muscle during atrophy and its recovery during the return of normal functional states. The observation of Fischer (6) that electrical stimulation delays the atrophy of denervated muscle is significant in this respect.

It should be pointed out that another basic factor may be common to the different types of atrophy. That is the effects of the loss, partial or complete, of neurohumoral agencies acting in a trophic manner, apart from the rôle they may play in the excitation processes associated with tension development. However, the finding that artificial stimulation of the muscle will arrest the weight loss and fall in glycogen concentration after the disappearance of viability in the severed nerves does not suggest any important rôle of such agencies other than that concerned with stimulus transmission.

SUMMARY

Experiments were carried out on the gastrocnemius muscles of adult albino rats. Denervation, immobilization by casts and tenotomy were followed in a few days by comparable marked falls in the glycogen concentration of the affected muscles. The glycogen changes occurred at a greater velocity than the loss of creatine, mass or strength from atrophic muscles. Conversely, restoration of muscle function through nerve regeneration, regrowth of tendon and cast removal was followed by a rapid and complete restoration of glycogen concentration to normal levels sooner than the recovery of creatine, mass or strength.

Adrenalectomy did not prevent the fall in glycogen concentration in denervated muscle.

Even though the fall in glycogen concentration and subsequent recovery coincides in time with the appearance and disappearance of fibrillary activity, it is believed that fibrillary contractions per se are not the cause of the low glycogen concentration in denervated muscle. The finding of equally marked and rapid decreases in the glycogen concentration of muscle undergoing atrophy without fibrillary activity, the fact that quinidine abolishes fibrillary activity without preventing the fall in glycogen and the observation that electrical stimulation will lessen rather than enhance the loss of glycogen from denervated muscle, indicate fundamental causes other than fibrillary activity.

It is suggested that the absence of tension development in the atrophic muscle results in insufficient energy levels for the coupled reactions which may be required for glycogen synthesis.

We are grateful to Dr. G. Clinton Knowlton for assistance in the fibrillary activity studies.

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POSTURAL CHANGES IN RESPIRATION[1]

ELIZABETH BROGDON FRANSEEN AND F. A. HELLEBRANDT

From the Departments of Physiology, Mount Holyoke College and the University of Wisconsin

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Dependence of respiration on body position was recognized by Liljestrand and Wollin in 1913. They demonstrated an increased ventilation during standing which they attributed chiefly to an alteration in respiratory rate. McMichael (1937) believes the lowering of alveolar CO_2 in the erect position found by him and others (Turner, 1927; Main, 1937) refutes the possibility that the increase in pulmonary ventilation is apparent only, secondary to a postural change in pulmonary capacity. Neither is the increased metabolism of standing adequate to account for the augmentation (Turner, 1927; Hamilton et al., 1932).

There are many indications that postural changes in respiration are related to the hydrostatic opposition offered the circulation in standing. Turner (1927) noted that overbreathing occurred especially among subjects who tolerated protracted standing poorly. This observation is supported by the postural studies of McMichael (1937) in which the pulmonary ventilation per 100 cc. of O_2 consumed varied approximately inversely as the absolute cardiac output. Hamilton et al. (1932) report that an increase in pulmonary minute volume became evident as their male subjects reached an angle of 50 degrees from the horizontal when supported on a tilting table and moved vertically by 10 degree increments. Approximately the same angle was found by Hellebrandt and Brogdon (1938) to be the critical position at which circulatory embarrassment commonly become evident in young women. This study is concerned with systematic observations of respiratory behavior under conditions of graded hydrostatic handicap to the circulation.

METHODS. The report deals in part with a further analysis of data obtained in a study of the postural metabolism of three young adult women (Hellebrandt et al., 1940). Pulmonary ventilation was determined from respiratory rate and amplitude as obtained in closed circuit indirect calorimetry. It was converted into liters per minute under standard conditions. To exclude the effect of oxidative processes on ventilation values, the "ventilation equivalent of oxygen" was calculated. The ratio, as used by Anthony (1930) and others (Knipping and Monerieff, 1932; McMichael, 1937), represents the quantity of air from which 100 cc. of O_2 are removed in the lung, or $\frac{PV \times 100}{\text{cc. } O_2/\text{min.}}$. It is similar to Simonson's "caloric ventilation equivalent" (1926) and Herbst's "utilization coefficient" (1928).

[1] Supported in part by the Wisconsin Alumni Research Foundation and the Council of Physical Therapy of the American Medical Association.

The three subjects of the major observations, young adult women, were under strictly basal conditions. After repeated 5-minute observations in recumbency, a series of determinations in some vertical or semi-vertical posture was made. Observations followed each other with a brief interruption after each 5-minute run during which pressure from the nose clip was momentarily relieved and the spirometer was partially refilled. As many as twelve consecutive runs could be made on a trained subject without restlessness or discomfort. The hydrostatic handicap was graded by the use of a tilting board. It was nullified by suspending the subject from the head and shoulders in a 180 gallon tank filled with water kept at body temperature. The full effect of gravity was observed in supported standing, with the subject immobilized by an arrangement of padded supporting bars, and in unsupported standing during which free movement above a stationary base was allowed as dictated by involuntary postural sway. Heart rates were counted for 30 seconds at minute intervals by pre-cordial auscultation. The experimental periods varied from 10 to 60 minutes in length.

The respiratory response to protracted support in the critical position (75 degrees from the horizontal) was studied in 22 healthy young adult women, well accustomed to the procedures. These experiments were not performed in the post-absorptive state but were basal in all other respects. Room temperature was held approximately at 75 degrees F. After horizontal observations were made, the subject was shifted to the semi-vertical position and rested motionless, leaning against the tilting table. Measurement of O_2 consumption was resumed within three minutes after the change in posture and was continued for 40 minutes or until the subject presented the signs of pre-collapse or actual syncope.

RESULTS AND THEIR INTERPRETATION. Two of the three subjects upon whom the most extensive data were obtained demonstrated marked respiratory alterations with the changes in posture. The third subject (R. H. T.) exhibited some increase in ventilation as the hydrostatic handicap to the cirulation was augmented. It was never marked. Observed differences were in some positions so slight as to be statistically unreliable. The more exaggerated respiratory activity of subjects F. A. H. and R. E. B. parallels their cardiovascular response to passive support at the 75 degree angle. F. A. H. could tolerate only 27 minutes in that position, with a sudden slowing of heart rate after an acceleration to 123 beats per minute preceding syncope. R. E. B. could tolerate 45 minutes without syncope, but only with a marked progressive increase in heart rate.

1. *Graded degrees of passive support.* A progressive increase in total ventilation for R. E. B. is evident in figure 1. It appears to be the result of an augmenting rate of breathing. Table 1 gives the mean of the values obtained at each angle of support for the three subjects. Treated statistically, the differences between the adjacent means from 15 degrees through 75 degrees were reliable throughout for R. E. B. and F. A. H. Respiratory amplitude was affected less consistently than was rate although deep inspirations and yawns did appear at the more vertical angles. The increasing ventilation equivalents (table 1) indicate that the magnitude of the respiratory change is out of proportion to

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concomitant increases in oxygen consumption. This is statistically true for the three subjects in most instances.

All the respiratory phenomena increase little further or fall off when the posture changes from 75 to 90 degrees from the horizontal. This was found to be true also in regard to the behavior of the heart, suggesting that muscle action consequent upon more active support of the body weight in full verticality exerts sufficient pressure upon the vascular walls to oppose in some measure a progressively greater accumulation of fluid in the dependent limbs due to the combined effects of stagnation and edema.

2. *Protracted support in a near-vertical position.* In the group of 22 young women there was a wide range in immediate response to change from the horizontal to a 75 degree angle. That the majority of the values for ventilation were in magnitude and direction influenced in the first 5 minutes by variations in oxygen consumption is indicated by the fact that in only four cases did the ventilation equivalent alter by more than 10 per cent. In these four, the ventilation equivalent increased between 16.9 and 43.1 per cent, evidence of definite over-ventilation.

TABLE 1

*Respiratory behavior of subjects supported at progressively more vertical angles**

SUBJECT		DEGREE OF DEVIATION FROM THE HORIZONTAL					
		15	30	45	60	75	90
F. A. H.	PV	6.87	7.44	8.37	10.06	12.10	11.68
	VE	3.75	4.01	4.53	5.14	5.84	5.66
	RR	19.90	22.78	24.94	24.75	28.95	25.55
	RA	346.82	328.00	337.15	411.15	420.80	450.95
R. E. B.	PV	6.06	7.29	7.93	8.37	9.29	9.59
	VE	3.42	4.10	4.22	4.65	5.12	5.14
	RR	15.10	19.22	23.05	23.04	25.25	25.17
	RA	402.30	381.32	345.31	366.70	372.40	384.73
R. H. T.	PV	5.74	6.19	6.33	6.47	7.22	7.10
	VE	2.93	3.18	3.28	3.27	3.44	3.47
	RR	12.20	13.40	14.17	13.69	14.30	14.90
	RA	474.70	464.20	451.60	474.70	509.90	478.60

* Values are the mean of from 8 to 12 five-minute determinations.
PV: Pulmonary ventilation in liters per minute.
VE: Ventilation equivalent (liters per 100 cc. of O_2 used).
RR: Respiratory rate per minute.
RA: Respiratory amplitude in cubic centimeters.

During the period of support at the 75 degree angle, pulmonary ventilation in 20 of the 22 subjects rather steadily increased. In nine cases the increase was due primarily to accelerated respiratory rate, in seven chiefly to greater amplitude, and in four to a combination of both factors. Fourteen of the subjects demonstrated increases so out of proportion to changes in metabolism

that final ventilation equivalents from 10 to 61 per cent higher than in the horizontal were obtained.

3. *Vertical postures.* Table 2 summarizes the data for all strictly vertical positions and figure 1 illustrates the response of R. E. B. With *suspension in water,* respiratory behavior approximates that in the horizontal position or is somewhat reduced. This suggests that the hydrostatic effect of gravity on the circulation is a factor which can modify respiration, for when it is nullified, the vertical position alone has no influence on ventilation. Undoubtedly, the withdrawal of proprioceptive stimuli and the exclusion of variable environmental influences contribute materially to the lessening.

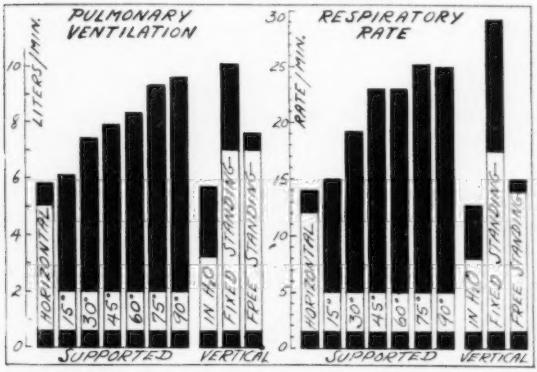

Fig. 1

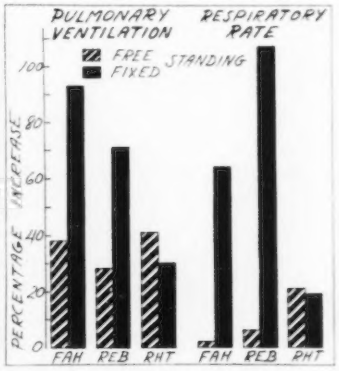

Fig. 2

Fig. 1. Respiratory response of one subject (R. E. B.) in supported (angle of tilt from the horizontal given), unsupported, and suspended postures. Columns are based on the means of 8 to 14 five-minute determinations in semi-vertical or vertical positions and are compared with the mean of 65 determinations in the horizontal.

Fig. 2. Comparison of effect on respiration of two types of vertical posture, showing percentage increase over horizontal values for pulmonary ventilation and respiratory rate. Columns are based on the means of 11 to 23 five-minute determinations and are compared with the mean of 93, 65 and 77 determinations for the three subjects in the horizontal.

Deprivation of postural sway in the erect position markedly augmented pulmonary ventilation in subjects F. A. H. and R. E. B. They showed, respectively, increases of 92.8 and 71.1 per cent over the horizontal values, figures definitely beyond any expected rise due to the metabolic cost of standing as is apparent from the significant increase in ventilation equivalents (table 2). The change is largely brought about by alteration in rate of breathing as illustrated in figure 2.

Standing comfortably and making no effort to restrict involuntary movement above the stationary base of support, subjects F. A. H. and R. E. B. exhibited mean pulmonary ventilation increases of 36.1 and 28.8 per cent over their horizontal values. The relatively slight change in ventilation equivalents indicates that such increases in ventilation are partially related to additional oxygen utilization. R. H. T. demonstrated comparatively little augmentation

of respiration in either posture and that amount is shown to be related to a concomitant rise in oxygen consumption. The ventilation equivalents do not differ significantly from those in the horizontal position.

Respiratory irregularities were prominent in the records of the two subjects who showed heightened respiration in the erect position. Deep inspirations and yawns were interspersed from one to eight times during a 5-minute period, more frequently in the rigid posture than when physiological sway was permitted. Deprived of sway, the subjects exhibited a periodicity in amplitude

TABLE 2

*Respiratory behavior in different vertical positions compared with that in the horizontal**

SUBJECT	F	VENTILATION EQUIVALENT, LITERS/100 CC. OF OXYGEN			PULMONARY VENTILATION, LITERS/MIN.			RESPIRATORY RATE PER MINUTE			RESPIRATORY AMPLITUDE, CC.		
		Mean	$\sigma_{dist.}$	$D/\sigma_{diff.}$	Mean	$\sigma_{dist.}$	$D/\sigma_{diff.}$	Mean	$\sigma_{dist.}$	$D/\sigma_{diff.}$	Mean	$\sigma_{dist.}$	$D/\sigma_{diff.}$
F. A. H.													
Basal	93	3.93	0.33		6.98	0.95		21.70	3.35		342.79	19.10	
Water	12	3.70	0.20	3.65	7.38	0.50	2.27	20.25	1.48	2.64	364.65	12.63	1.98
Fixed	11	7.14	1.32	8.07	13.66	2.19	10.01	35.70	4.70	9.60	388.86	38.86	3.65
Sway	23	4.49	0.88	3.04	9.50	0.78	13.33	22.08	0.96	0.95	430.10	27.31	14.49
R. E. B.													
Basal	65	3.36	0.27		5.89	0.28		14.16	1.30		419.48	39.00	
Water	10	2.90	0.19	6.57	5.70	0.22	2.47	12.76	1.14	3.54	449.80	33.41	1.98
Fixed	12	5.43	0.58	12.18	10.13	1.30	11.28	29.28	4.92	10.59	448.22	15.67	4.34
Sway	14	3.57	0.31	2.36	7.63	0.59	10.81	15.09	2.10	1.60	509.84	54.23	5.91
R. H. T.													
Basal	77	3.07	0.31		5.79	0.54		11.63	1.75		504.69	61.92	
Water	11	2.61	0.16	8.52	5.79	0.39	0	12.03	1.30	0.91	486.10	58.60	0.98
Fixed	12	3.37	0.40	2.54	7.51	0.55	10.12	13.90	1.39	5.07	541.67	40.61	2.72
Sway	12	3.28	0.40	1.78	8.15	0.76	10.31	14.18	1.84	4.50	586.37	34.18	6.72

* Water: Suspended in 180 gallon tank of water.
Fixed: Immobilized in standing position by extrinsic supports.
Sway: Free postural sway unrestrained.
F: Number of 5-minute periods of observation.
$D/\sigma_{diff.}$: Comparison of mean of that position with the horizontal basal mean. It is customary to take a $D/\sigma_{diff.}$ of 3 as indicative of a significant difference.

occasionally so marked as to resemble Cheynes-Stokes respiration. Syncope when it occurred always followed one of these exaggerated phases.

COMMENT. Respiratory movements are sometimes considered as a subsidiary line of defense against orthostatic collapse by virtue of expiratory compression of the abdomen and inspiratory thoracic suction (Best and Taylor, 1939). Hill and Barnard in 1897 showed that when splanchnic vasomotor tone of their animals was destroyed, respiratory efforts maintained the circulation, though far less efficiently. On the basis of plethysmographic experiments with dogs

in 1933, Eyster and Hicks believe that the influence of the extent of breathing on venous return is not as great as is ordinarily stated. More recently, Boyd and Patras (1941), using a cardiometer in the closed chest, have reported increases in diastolic and stroke volume with inspiration which were exaggerated by the deep and prolonged breathing following vagotomy.

In our experiments it is impossible to evaluate the importance of this respiratory pump as an aid to the circulation. Respiratory behavior is shown to be closely related to cardiovascular competence. It is augmented as the gravitational handicap to the circulation is made greater and approaches normal when the compensatory action of postural sway reduces that handicap. With few exceptions both fainters and non-fainters responded with some augmentation of ventilation to protracted support in near-verticality. However, the point of failure of the circulation did not appear to bear any time relationship to the percentage increase in ventilation. Whether the increase in breathing prolongs the period of tolerance for the erect position through aspiratory action can only be a matter of conjecture. The fact that the increases observed were in many cases due to rises in rate with reductions in ampliture renders this unlikely. The augmented ventilation seems rather a reflection of the general slowing of the circulation in which the medullary centers are more or less seriously deprived of oxygen unless more effective compensatory mechanisms combat the pull of gravity upon the blood column. Of these, the rhythmic squeezing action of muscle is clearly important.

CONCLUSIONS

1. Respiratory changes induced by alteration of posture outstrip concomitant metabolic increases as verticality is approached.

2. Increases in ventilation in vertical postures appear to be secondary to interference with blood flow to the head.

3. Postural sway is a factor of material benefit in combating orthostatic hyperpnea.

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LIVER FUNCTION, PULSE RATE AND TEMPERATURE OF HYPERTHYROID DOGS

Effect of a yeast-free diet and a high B vitamin diet[1, 2]

VICTOR A. DRILL, C. BOYD SHAFFER and RICHARD OVERMAN

From the Section of Physiology, Biological Laboratories, Princeton University, Princeton, N. J.

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Previous studies on the liver function of dogs during hyperthyroidism have shown that a deficiency of the B vitamins is related to the production of an abnormal liver function. It was found that the removal of the yeast from the diet of hyperthyroid dogs was followed by the sudden appearance of abnormal liver functions, as judged by the excretion of injected bromsulphalein (1, 2). Once an abnormal liver function had been produced in hyperthyroid dogs by removal of the yeast from the diet, intensive treatment with crystalline vitamin B_1 (thiamine) and a yeast concentrate would not restore the liver function to normal (2).

Thyroid feeding also produced a marked tachycardia, but on removal of the dietary yeast a rapid fall in pulse rate was observed, the pulse rate falling to as low as 80 beats per minute while still feeding thyroid gland (2). The injection of crystalline thiamine would raise the pulse rate to the hyperthyroid level in 24 to 48 hours. While the mechanism of this effect of thiamine on pulse rate during hyperthyroidism is unknown, it is evident that the tachycardia observed in experimental hyperthyroidism depends not only on thyroid feeding per se but also on an adequate intake of thiamine. The treatment of these dogs with crystalline thiamine and a yeast concentrate would maintain the pulse rate at the previous hyperthyroid level, but after 90 to 100 days of thyroid feeding the pulse rate again began to fall toward normal. The pulse rate remained above normal, but not at the previous hyperthyroid level (2).

Further information on the interrelationships between the B vitamins and the thyroid gland was obtained by studying *a*, the effect of thyroid feeding on the liver function and pulse rate of dogs receiving a diet rich in the B vitamins from the start of thyroid feeding; *b*, the effect of thyroid feeding on the liver function and pulse rate of dogs receiving a vitamin-B-complex-free diet.

Methods. As a sex difference in response to thyroid feeding has been reported in rats (3), only male dogs were used. Thyroid gland[3] was fed in a dosage of

[1] The authors wish to thank Eli Lilly and Company for a grant in support of these experiments.

[2] This work was done in the laboratory of Dr. W. W. Swingle and we are indebted to him for the necessary facilities to undertake this work.

[3] The authors are indebted to Dr. C. N. Fry of The Fleischmann Laboratories for supplying the yeast concentrate, to Dr. R. T. Major of Merck and Company for supplying the crystalline thiamine, and to Dr. H. W. Rhodehamel of Eli Lilly and Company for the desiccated thyroid gland.

0.4 or 0.6 gram per kilogram of body weight per day (table 1) throughout the experiment. The dogs were fed a modification of Cowgill's casein diet no. III which is free of the B vitamins (1). The animals were fed at the same time each day, being allowed to eat as much as they wanted for a three hour period, and the food intake measured. Control measurements were made for a period of 3 to 4 weeks, using a yeast supplement to the basal diet, until the food intake became fairly constant. Liver functions were determined by the bromsulphalein method of Rosenthal and White as previously described (2), using 5 mgm. of dye/kgm. body weight.

Four of the dogs were fed 10 grams of a yeast concentrate[3] per day, the yeast containing 200 U.S.P. units of vitamin B_1, 230 gamma of riboflavin, 200 gamma of vitamin B_6, and 1500 to 2000 gamma of calcium pantothenate per gram. Two of these dogs also received a daily injection of 1.0 mgm. of thiamine each day. The administration of the yeast concentrate and thiamine began with the feeding of thyroid gland and was continued throughout the experiment.

Three other dogs were fed thyroid gland, but at the start of thyroid feeding

TABLE 1

DOG NO.	WEIGHT	THYROID GLAND FED PER DAY PER KGM. BODY WEIGHT	YEAST CONCENTRATE FED PER DAY	THIAMINE INJECTED PER DAY
	kgm.	*grams*	*grams*	*mgm.*
1	16.1	0.6	10	0
2	8.8	0.6	10	1.0
3	10.1	0.4	10	1.0
4	12.8	0.4	10	0
5	10.3	0.4	0	0
6	8.6	0.6	0	0
7	10.8	0.6	0	0

the yeast was removed from the diet, and they were fed the yeast-free diet throughout the period of thyroid feeding.

RESULTS. *Liver function.* The control measurements of liver function did not show a retention of dye greater than 12 per cent at the end of 30 minutes. A dye retention of 15 per cent or greater was therefore regarded as abnormal. Of the four hyperthyroid dogs receiving the high yeast diet, dog 3 first showed an abnormal liver function on the 73rd day of thyroid feeding. Later, an abnormal liver function developed in dogs 2, 4 and 1 on the 84th, 91st and 112th day of thyroid feeding respectively (fig. 1). Figure 1 shows the time and degree of dye retention when an abnormal liver function was first observed. The dye retention of the dogs later increased and remained between 30 to 50 per cent retention of dye. No difference was observed in the production of the abnormal liver functions between the dogs fed 0.4 or 0.6 gram of thyroid per kilogram of body weight. The amount of B vitamins supplied to these dogs was sufficient to maintain food intake and prevent a loss of body weight. Included in figure 1 for comparison are three dogs fed the same diet but receiving

only a small amount of yeast (2). The amount of yeast fed (basal yeast) is sufficient for normal maintenance, but when the requirements for the B vitamins are increased by thyroid feeding a relative deficiency of the B vitamins is produced. The thyroid-fed dogs receiving basal yeast supplements developed an abnormal liver function in an average of 45 days, whereas the hyperthyroid dogs fed a high B vitamin diet showed abnormal liver functions after an average of 90 days. This demonstrates, therefore, that feeding hyperthyroid dogs a diet rich in the B vitamins will delay the onset of an abnormal liver function, as judged by the bromsulphalein test, but will not prevent its appearance.

The hyperthyroid dogs receiving a yeast-free diet showed a marked loss of appetite and weight and rapidly developed abnormal liver functions (figs. 1, 4), the average time being 22 days for the first appearance of abnormal dye reten-

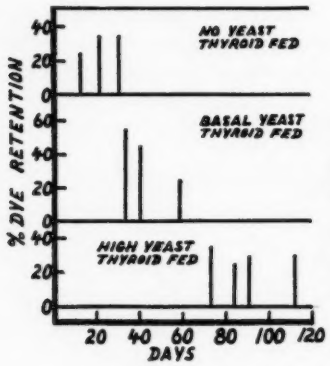

Fig. 1

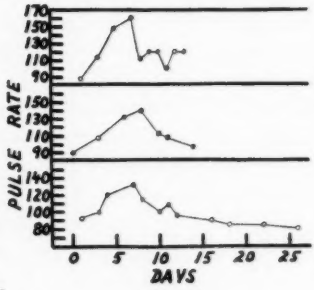

Fig. 2

Fig. 1. Liver functions of hyperthyroid dogs fed various amounts of B vitamins. The graph illustrates the time at which an abnormal liver function was first observed in each dog and the percentage of dye retention at that time.

Fig. 2. The pulse rate of hyperthyroid dogs fed a yeast-free diet. Note the rapid rise in pulse rate which returns to normal within 10 days of thyroid feeding, as soon as the body stores of thiamine are depleted.

tion. Thus the feeding of a diet free of the B vitamins renders the dogs extremely susceptible to the effects of thyroid feeding, producing an abnormal liver function in a short time.

Pulse rate. It has previously been shown that the tachycardia typically seen in experimental hyperthyroidism cannot be maintained if the yeast is removed from the diet after about 30 to 50 days of thyroid feeding (2). An injection of vitamin B_1 (thiamine) will raise the pulse rate to its previous hyperthyroid level (2). Confirmation of this result was obtained when the yeast was removed from the diet at the time thyroid feeding was started. When this was done only a short, rapid rise in pulse rate was obtained, which returned to normal during the first 10 days of thyroid feeding (fig. 2). The small initial rise in pulse rate is due to the presence of the body stores of thiamine, which were soon

exhausted, and the pulse rate declined to normal, even while still feeding thyroid gland. Thus, on a diet free of the B vitamins, thyroid feeding produced only a temporary rise in pulse rate, which returned to normal in 10 days. The tachycardia seen in experimental hyperthyroidism is therefore not due to thyroid feeding per se, but also depends on an adequate supply of thiamine (2).

Earlier studies have shown that the low pulse rate produced in hyperthyroid dogs by removing the yeast from the diet can be raised to its previous hyperthyroid level by injecting thiamine (2). It was also observed that intensive treatment with thiamine and yeast concentrate maintained the tachycardia,

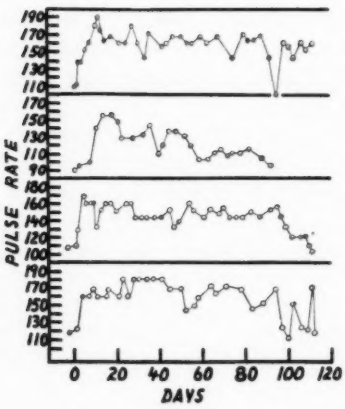

Fig. 3

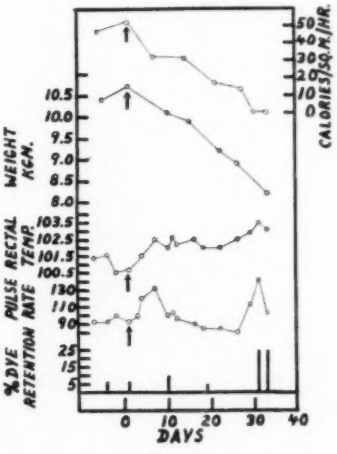

Fig. 4

Fig. 3. The pulse rate of hyperthyroid dogs fed a high B vitamin diet, on which the tachycardia produced by thyroid feeding cannot be maintained indefinitely. The fall in pulse rate towards the end of the experiment seems to be associated with a compensatory hypertrophy of the heart.

Fig. 4. Composite graph of dog 7 receiving thyroid and a yeast-free diet. Note the rapid loss of appetite and weight, the continuous rise in rectal temperature, and a terminal rise in pulse rate. The food intake is calculated as calories consumed per square meter of surface area per hour.

but only for 90 to 100 days, after which time the pulse rate began to fall towards normal (2). This was explained as probably being due to a compensatory hypertrophy of the heart. This was further studied in the dogs that were fed a diet rich in the B vitamins at the time thyroid feeding was started. The results (fig. 3) showed that the tachycardia in hyperthyroid dogs can not be maintained indefinitely by such treatment. Three of the dogs showed a drop in pulse rate as the thyroid feeding was continued. The fourth dog would probably have shown a similar drop in pulse rate had the thyroid feeding been continued longer.

At necropsy the heart of the hyperthyroid dogs receiving the high B vitamin

diet was found to be hypertrophied, particularly the left ventricle. The heart of these dogs weighed an average of 97.7 grams per 10 kilos of body weight as compared with 70.8 grams per 10 kilos of body weight of normal dogs (table 2). The hyperthyroid dogs receiving a yeast-free diet also showed some cardiac hypertrophy, the heart weight being 85.0 grams per 100 kilos of body weight. These dogs, however, were fed thyroid gland for a shorter period of time.

Rectal temperature. The rectal temperature of hyperthyroid dogs receiving a high yeast diet was maintained at a level above normal throughout the experiment. Dog 4 showed an increase in rectal temperature from a normal of 101.0°F. to 102–102.5°F. after thyroid feeding was started. Likewise dog 3 increased from a normal of 100.5–101.0 to 102.0°F. and dog 1 from 101.5 to 102–102.5°F. These high temperatures were maintained fairly constant throughout the experiment. Dog 2 was not as constant. His rectal temperature rose from 101.5 to 102–102.5°F., returning at intervals to normal.

The hyperthyroid dogs fed a yeast-free diet showed a greater rise in rectal

TABLE 2

Weight of heart of hyperthyroid dogs

(Grams per 10 kilos of body weight)

DOG NO.	HYPERTHYROID HIGH YEAST DIET	DOG NO.	HYPERTHYROID YEAST-FREE DIET	DOG NO.	NORMAL DOGS
1	80	5	72	8	70
2	100	6	94	9	70
3	93	7	86	10	59
4	76			11	78
				12	77
Average.....	97.7		85.0		70.8

temperature (fig. 4). A marked terminal rise in temperature was also seen as the dogs developed anorexia and lost weight. A terminal rise in pulse rate was also seen in one dog (fig. 4).

Discussion. Thus far the B vitamins have been shown to bear a causal relationship to the production of an abnormal liver function in hyperthyroid dogs (1, 2). After an abnormal liver function has been produced in hyperthyroid dogs it cannot be brought again to normal by intensive treatment with thiamine and yeast concentrate (2). In this paper it was shown that an abnormal liver function develops very rapidly, in an average of 22 days, when thyroid-fed dogs receive a yeast-free diet. Hyperthyroid dogs fed a basal amount of yeast each day developed an abnormal liver function in an average of 45 days. In other hyperthyroid dogs receiving a high B vitamin diet it took an average of 90 days before an abnormal liver function was produced (fig. 1). This demonstrates that a direct relationship exists between the amount of B vitamins in the diet of a hyperthyroid dog and the time elapsed before an abnormal liver function develops. With a diet rich in the B vitamins the pro-

duction of an abnormal liver function can be greatly delayed, but cannot be prevented. Enough B vitamins were administered to these dogs to prevent a loss of weight and to maintain the food intake at the hyperthyroid level. Whether a greater intake of the B vitamins would further have delayed the appearance of an abnormal liver function is not known. It may be said, however, that the development of an abnormal liver function does not depend on a loss of weight. In order to maintain body weight, hyperthyroid dogs fed yeast concentrate and injected with thiamine voluntarily eat approximately 1.5 times as many calories per day as do normal dogs (4). It is a possibility that the increased food intake, which in turn will provide more metabolic work for the liver, is a factor in itself which will tend to produce an abnormal liver function. Unpublished work on serum phosphatase has shown that the phosphatase remains normal while dye retention is normal, and that an increase in phosphatase is only obtained when the dye retention is abnormal.

Bartels (5) has reported that in hyperthyroid patients no correlation was found between the duration of the disease and the incidence of abnormal liver functions. Rather, a normal liver function was generally obtained in patients who had no previous history of a loss of weight. Judging from the work on hyperthyroid dogs it is possible that the hyperthyroid patients not showing a weight loss were receiving a greater supply of the B vitamins, which would also aid in maintaining a normal liver function. Further references on liver function in Grave's disease may be found in Boyce's recent monograph on the liver (6).

The effect of the high B vitamin diet in delaying the appearance of the abnormal liver function in hyperthyroid dogs is in agreement with other investigations. It has been reported that the administration of yeast will protect rats against the appearance of hepatic cirrhosis produced by p-dimethyl-amino-azo-benzol (7). Von Glahn and Flinn found that yeast would partially protect the liver of rabbits against the effect of feeding lead arsenate (8). Patek and Post (9, 10) obtained beneficial effects in cases of human cirrhosis by feeding a nutritious diet supplemented by vitamin B concentrates, but could not find any protective effect of yeast on experimentally produced carbon tetrachloride cirrhosis in rats (11). They found however, that rats receiving 8 to 11 grams of food per day had more severe hepatic lesions than rats fed 14 grams per day. In our experiments on hyperthyroid dogs the animals receiving the high B vitamin diet, with which it took an average of 90 days to produce an abnormal liver function, ate approximately 1.5 times as much food as normal. This increased voluntary food intake is necessary (4, 17), and was sufficient, to prevent a loss of weight in the hyperthyroid dogs. Thus, this increased food intake is a possible factor in delaying the appearance of an abnormal liver function. If this is so, it is still necessary that the high B vitamin diet be supplied if the food intake is to be increased.

Pulse rate. As previously noted (2, 12), the cardiac symptoms of beri-beri and hyperthyroidism in humans are quite similar. In man tachycardia is produced in both diseases. Thus a deficiency of thiamine would be expected to intensify any tachycardia produced by Graves' disease. However, in the dog a

deficiency of thiamine tends to produce bradycardia. When the yeast is removed from the diet of a hyperthyroid dog the pulse rate rapidly falls to normal and can be raised to its hyperthyroid level by an injection of thiamine (2). In Graves' disease the administration of thiamine should tend to reduce the pulse rate if any degree of a thiamine deficiency previously existed. Frazier and Ravdin (12) have observed a reduction in the pulse rate of hyperthyroid patients treated with thiamine and yeast. The studies in this paper have shown that when the dietary yeast is removed at the time thyroid feeding is started, only a slight tachycardia is obtained which returns to normal within 10 days. This again shows that production of tachycardia in thyroid-fed dogs depends on an adequate supply of vitamin B_1. If thiamine and yeast are administered to hyperthyroid dogs who have had the yeast removed from their diet the pulse rate will rise to the previous hyperthyroid level, but despite this treatment the pulse rate will return towards normal if thyroid feeding is continued for 80 to 100 days. Likewise, the pulse rate of hyperthyroid dogs receiving a high B vitamin from the start of thyroid feeding also begins to return to normal after a similar period of thyroid administration. The fall in pulse rate at this time is associated with a hypertrophy of the heart (table 2).

The evidence in the literature demonstrates that the B vitamins play an important rôle in the symptoms and changes produced by feeding thyroid gland (1–4, 13). There is also some clinical evidence to support this view (12, 14, 15). It should be emphasized that the B vitamins will not counteract the effects of hyperthyroidism per se, either experimental or clinical, but will only effect such symptoms as are produced by a deficiency of these B vitamins, the deficiency of the B vitamins, or an increased requirement for, being in turn produced by thyroid feeding (16, 17).

CONCLUSIONS

1. A yeast-free diet renders dogs extremely susceptible to the effects of thyroid feeding, an abnormal liver function being produced in an average of 22 days. Thyroid-fed dogs receiving a small amount of yeast each day developed an abnormal liver function in an average of 45 days. When hyperthyroid dogs were fed a high B vitamin diet it took an average of 90 days before an abnormal liver function was observed. Thus, the amount of B vitamins fed has a direct relationship to the time elapsed before an abnormal liver function develops. A high B vitamin diet will delay but will not prevent the appearance of an abnormal liver function in hyperthyroid dogs.

2. Hyperthyroid dogs fed a yeast-free diet develop only a slight tachycardia which returns to normal within 10 days. Thus, in an absence of thiamine (vitamin B_1) thyroid feeding does not produce tachycardia.

3. Hyperthyroid dogs receiving a high B vitamin diet maintained a high pulse rate for about 80 to 100 days of thyroid feeding, after which time the pulse rate fell toward normal. The drop in pulse rate at this time seemed to be correlated with a compensatory hypertrophy of the heart.

4. The relation of these changes to those seen in Graves' disease is discussed.

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EFFECT OF ULTRAVIOLET RADIATION ON BODY WEIGHT OF MICE

HAROLD F. BLUM, HUGH G. GRADY AND JOHN S. KIRBY-SMITH

From the National Cancer Institute, National Institute of Health, United States Public Health Service

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The following observations on body weight were made in the course of investigations of the carcinogenic action of ultraviolet radiation. Large numbers of animals were involved in these studies; in fact more than might justifiably be devoted to an investigation dealing solely with the present topic. Hence the measurements obtained are highly dependable, but on the other hand the experimental attack has not been as direct as had this been an independent study.

EFFECT OF MERCURY ARC RADIATION. *Method.* The method of dosage has been discussed elsewhere (Blum, Kirby-Smith and Grady, 1941), and only a brief description will be necessary here. The source of radiation was an intermediate pressure mercury arc enclosed in quartz. The amount of radiation of wavelengths shorter than 3200A[1] emitted by this lamp was measured continuously throughout the period of exposure by means of a titanium photocell and integrating recorder. Thus, dosage and intensity of radiation were accurately controlled.

Two to three months old inbred, albino mice (strain A) were used. They were given a diet of Purina dog chow *ad libitum* except in one experiment where diet was purposely restricted. Each experimental group was made up of 26 animals at the beginning. Average body weights were obtained from weekly group weighings. The standard deviation for individual body weights, determined for several of the groups was approximately 10 per cent of the group mean. At higher doses some of the animals developed tumors after about 110 days and were killed for study; hence the average body weights are less reliable after this time.

Dose-body weight relationship. Figure 1 shows average body weights of several groups of mice each receiving five exposures to ultraviolet radiation per week. A relative decrease in average body weight of exposed as compared with control animals is clearly shown, this effect being greater at higher dosages. Lower body weight for animals exposed to mercury arc radiation as compared to control animals was reported by Ellinger (1938, 1939) whose observation is amply confirmed by these findings.

The relationship between body weight and dosage is illustrated in figure 2. For comparison, four of the weekly weighings have been averaged, namely, those of the 16th to 19th weeks after irradiation began. This is a good period for comparison since the group body weight becomes approximately constant at this time, yet the incidence of tumors is still low. The essential relationship

[1] See Blum, Kirby-Smith, and Grady (1941) for the spectral relationships involved. The importance of this wavelength limit will become apparent in later discussion (p. 380).

is not altered if another period is chosen for the comparison. Some of the points in the figure were obtained by averaging weights of two or more groups that received the same dose.

The relationship between body weight and logarithm of dose is linear down to a certain dosage below which body weight does not vary with dose; this value is somewhat different for each experiment. In experiments II and IV (fig. 2) the broken lines represent body weights of control groups. In experiments I and III no controls were maintained, but the constancy of body weight below a given dosage clearly indicates this as the control weight. The differences in body weight among the control and low dosage groups probably represent group and seasonal differences. Experiments I and II were practically concurrent whereas III and IV were carried out at another season. All the data indicate

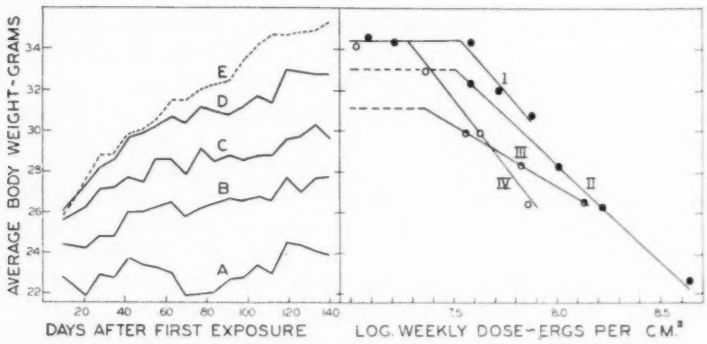

Fig. 1 Fig. 2

Fig. 1. Average body weights of mice exposed to different dosages of radiation from an intermediate pressure mercury arc. Weekly doses as follows: A, 43.0×10^7; B, 16.5×10^7; C, 9.9×10^7; D, 3.6×10^7 ergs per cm² of wavelengths shorter than 3200A, E, control. The weekly dose was given in this experiment in equal exposures on five days of the week.

Fig. 2. Relationships between body weight and dosage of radiation. In experiments I and II the weekly dose was given in five equal exposures per week; in III in seven equal exposures, and in IV in a single weekly exposure. The broken lines represent control weights.

that doses below a critical value do not affect body weight. There is no evidence that increase in body weight is stimulated by any dosage.

Three different schedules of dosage were used. In experiment IV the total weekly dose was given on one day per week, in experiments I and II it was divided into five equal exposures administered on five days, and in III into seven equal exposures, administered daily. The interval between doses affects the results quantitatively but does not alter the essential relationships. In general, larger doses given at longer intervals are more effective than smaller, more frequent doses. Destruction of tissue is also more marked under the former conditions (see p. 383).

If the exposures are interrupted the average body weight of the group increases rapidly, and may soon reach that of the controls.

Food intake. In one experiment the food consumption of irradiated and control animals was followed daily, and found to be distinctly less for the former. Paired feedings were not carried out, but after the experiment had continued for some weeks the food consumption of the controls was restricted to approximately that of the irradiated animals with the result that the body weight of the controls fell rapidly, almost to that of the irradiated mice. Thus, depression of body weight by ultraviolet radiation appears due to decreased food consumption rather than to qualitative modification of metabolism. The decreased food intake is accompanied by, and may result from, a general decrease in activity of the mice. It was noticeable that fighting among the animals decreased with increase in dosage of radiation, the irradiated mice being quieter and better groomed than the controls.

These facts throw doubt upon the hypothesis of Ellinger (1938, 1939) that the decreased body weight is due to increased thyroid gland activity indirectly stimulated by histamine produced in the skin by ultraviolet radiation. We have observed no gross or microscopic changes in this gland.

Effect of intensity. In some experiments the intensity of the radiation was varied, but constant dosage maintained. When the intensity was varied tenfold no differences in body weight were observed. In two groups each of fifty animals, in which a twenty-fold difference in intensity was maintained, the body weight was 3.5 per cent less for the group receiving the highest intensity. This suggests a slightly lower effectiveness for the lower intensity, but the difference is not statistically significant. Thus, the reciprocity law (Intensity $\times$ time = a constant) must hold within rather wide limits.

The effect of confinement of the animals in small cages during the period of exposure is shown by this observation to be unimportant since those groups receiving low intensities were confined much longer than those receiving high intensities.

THE ACTION SPECTRUM. In certain experiments the wavelength range was restricted by interposing Corex D, Pyrex, or window glass filters between the source and the animals. The window glass filter cuts off virtually all energy of wavelength 3200A and shorter. Mercury arc radiation passing through this filter does not affect body weight whereas that passing the Pyrex filter, which is confined principally to the 3130A line, does. Hence the long wavelength limit for this effect must lie between the adjacent 3130A and 3340A lines of the mercury arc. For this reason, wavelengths longer than 3130A are disregarded in the following tables.

Table 1 shows the intensities of the lines of the mercury arc incident upon the mice when no filter is interposed. These are expressed in ergs per cm.^{2} per second per line. The dosage, expressed in ergs per cm.^{2} is obtained by multiplying the intensity by the number of seconds of exposure. The transmissions of these lines by the Corex D and Pyrex filters are also given in this table. For a given line, the product of the intensity and the transmission gives the intensity incident on the mice when the particular filter is used. The sum of these values for all the lines, divided by the sum of the intensities of all the lines when no filter is

interposed, multiplied by the number of seconds of exposure gives the dosage incident through the filter.[2]

An experiment was also carried out with a low pressure mercury arc, which emits over 90 per cent of its energy as wavelength 2537A. About four per cent of the remainder is in the 3130A, 3020A and 2967A lines, but any effect of these was compensated by exposing the control for this group to the radiation from this source passing through a Corex D filter. By this arrangement the control did not receive wavelength 2537A, but virtually all the radiation of other wavelengths.

Table 2 summarizes the results of these experiments. Due as much to good fortune as design, the same reduction in body weight from that of the controls (6 per cent) was observed with the Pyrex and Corex D filters and with the 2537A

TABLE 1

Spectral data

WAVELENGTH λ	INCIDENT ENERGY WITHOUT FILTER (<λ3200A)	FRACTION OF INCIDENT ENERGY TRANSMITTED BY:		
		Corex D filter	Pyrex filter	Irradiated epidermis*
	ergs cm^{-2} $sec^{-1} \times 10^3$			
3130A	13.2	0.76	0.40	0.28
3022A	6.8	0.64	0.12	0.23
2967A	3.8	0.52	0.08	0.17
2925A	0.5	0.45		0.14
2894A	1.2	0.40		0.08
2804A	2.4	0.17		0.02
2752A	0.8	0.10		0.02
2699A	0.8	0.05		0.02
2652A	4.8	0.02		0.02
2602A	0.2			0.02
2537A	5.3			0.02

TABLE 2

SOURCE	FILTER	WAVELENGTHS	WEEKLY DOSE (WAVELENGTHS <3200A)		REDUCTION IN BODY WEIGHT BELOW THAT OF CONTROLS
			Incident on surface	Incident beneath epidermis	
			ergs cm^{-2} $\times 10^7$	ergs cm^{-2} $\times 10^7$	per cent
Intermediate pressure mercury arc	Window glass	>3130A	0	0	0
	Pyrex	>2967A	8.0	2.1	6
	Corex D	>2652A	4.1	1.0	6
	None	>2400A	5.3	0.8	6
Low pressure mercury arc	None	2537A	36.0	0.7	6

* The method for obtaining these values is described by Kirby-Smith, Blum and Grady (1942).

line. For comparison, the dose without filter which would bring about the same per cent reduction in body weight was estimated by interpolation from data obtained from a number of experiments. Thus the fourth column of the table presents the amounts of energy of various wavelength regions required to bring about a six per cent reduction in body weight.[3]

Whatever the inherent inaccuracies of these measurements, it is obvious that there is a great difference in the effectiveness of different wavelengths, when

[2] Actually the total energy rather than the time of exposure was measured in these experiments (Blum, Kirby-Smith and Grady 1941), but the essential principle of the calculation is the same.

[3] In making such a comparison it should be kept in mind that reduction in body weight varies as the logarithm of the dose.

considered in terms of energy incident on the skin surface. Wavelength 2537A is weakly effective, over four times as much energy being required to achieve the same effect as for wavelength 3100A (Pyrex filter), and nine times as much as for the radiation passing through the Corex D filter.

If changes in living cells are assumed to underlie this phenomenon, the dead corneum of the epidermis must be regarded as a screen which protects the underlying viable layers. The corneum displays selective absorption and must modify the relative effectiveness of the various wavelengths. This is an important factor in determining the character of the action spectrum for the erythema of sunburn of man, which is an analogous process.

An estimate of the magnitude of this effect can be made from measurements of the transmission of the epidermis, since the corneum is responsible for most of the absorption by this layer. Table 1 gives transmission values for the epidermis of the ear of an albino mouse, which had been subjected to about the same amount of radiation as those here compared, and these values have been used in calculating the energies incident beneath the epidermis which appear in column five of table 2. The different wavelengths seem to have nearly the same effectiveness when considered in terms of energy reaching the epidermis.

While these data do not provide an accurate action spectrum, they permit the conclusion that the corneum is not involved in the photochemical processes leading to the reduction of body weight. This helps to rule out the possibility that vitamin D plays a rôle in this phenomenon, since this substance must be formed chiefly in the superficial layer (see Blum, 1942). Wavelength 3130A is almost without effect in the production of vitamin D, whereas 2537A is quite effective (Bunker and Harris, 1937; Knudson and Benford, 1938), and this is the reverse of the relative effectiveness of these wavelengths in reducing body weight. This accords with Blum and Lippincott's (1942) failure to find evidence of hypervitaminosis D among these mice.

The long wavelength limit at 3200A places the action spectrum in that spectral range which produces damage to cells in general, among which effects the changes underlying the erythema of sunburn of man may be included. It is probable that protein (see Mitchell, 1938; Blum, 1941) is the substance primarily affected in this destructive reaction. Nucleic acid seems the only alternative substance, and in either case the long wavelength limit would have to be approximately that found experimentally. The falling off of the absorption spectra of these substances toward the long wavelength limit could account for the lower effectiveness of wavelength 3130A.

In recent years it has been shown that light influences the sexual cycle of certain mammals, and hence might affect metabolism and body weight. Such effects are brought about by wavelengths longer than 3200A (approximately those of the visible spectrum), however, and could not be responsible for the reduction of body weight found in the present experiments.

The effects of longer wavelengths on growth found by Luce-Clausen and Brown (1939) could not play a significant rôle under the experimental conditions we have used.

PATHOLOGICAL FINDINGS. Grossly, such radiation causes changes in the parts not covered by hair, namely, the ears, tails, paws, snouts, eyelids and eyes. The hair itself may display a yellow color after severe dosage, but the skin beneath the hair is little affected if at all. The ears show the greatest change, and have been subjected to the most thorough histological studies because they are the site of the great majority of tumors (Grady, Blum and Kirby-Smith 1941). Prior to the appearance of tumors, i.e., during the period body weight comparisons were made, the ears show changes varying with the dose from the mildest hyperplasia of the epidermis and deeper tissues to severe inflammation and necrosis. The magnitude of the change in body weight parallels the amounts of damage to the skin of the ears.

No pathological changes in internal organs, either gross or miscroscopic, were found which could be correlated with the external changes or with body weight effects. No abnormalities of the thyroid glands were observed.

DISCUSSION. It seems clearly established that ultraviolet radiation of wavelength shorter than 3200A slows the normal gain, and may even cause a decrease in body weight of mice. This is directly traceable to decreased food consumption, and parallels damage to cells of the skin. The interrelations of these events are obscure. While the hypothesis that toxic products (possibly histamine-like substances as suggested by Ellinger (1938, 1939)) resulting from cell damage underlie the decreased food intake seems most reasonable, direct proof is lacking and systemic changes that might be expected to result, have not been found. Other explanations might be offered, but failing supporting evidence prolonged discussion is not justified. Qualitative changes in metabolism are not demonstrated, and the absence of evidence of increase in body weight at any dosage is striking when one considers that benefits to general health are so commonly claimed for exposure to ultraviolet radiation. Caution must be exercised in applying these findings to man, however, for direct quantitative comparison cannot be made because of differences in transmission characteristics of the epidermis.

SUMMARY

Ultraviolet radiation of wavelengths shorter than 3200A slows normal gain or may reduce the body weight of mice. Below a certain dosage body weight is not affected. There is no evidence of growth stimulation at any dosage.

The extent to which body weight is affected varies with the total dosage and interval between doses, but within the limits explored intensity of the radiation is not a factor.

The lowering of body weight seems due to decreased food consumption.

Wavelength studies indicate that the radiation directly affects the living tissues of the skin, the corneum acting as a semi-opaque screen which protects the deeper layers.

The lowered body weight parallels destructive changes in the skin, but evidence of important changes in internal organs is lacking.

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THE EFFECTS OF VITAMIN D AND OTHER STEROLS ON BLOOD PRESSURE IN THE RAT[1]

H. L. BRISKIN,[2] F. R. STOKES, C. I. REED AND R. G. MRAZEK

From the Department of Physiology, University of Illinois, Chicago Colleges

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The earlier literature on toxic responses to vitamin D_2, has been reviewed repeatedly (1, 2, 3, 4). The effects on the kidney and on the vascular system have received considerable attention. Appelrot (5) reported that toxic doses of vitamin D_2 produced hypertension in dogs and suggested similarity between this action and that of adrenalin. He believed the hypertension to be due to hypertrophy of the media of the arterioles.

Goormaghtigh and Handovsky (6) also have found hypertension in dogs, with hypertrophy of medial muscle fibers in the arterioles of the kidneys, spleen and intestines, often followed by atrophy. Also, there was increased sensitivity to adrenalin.

The hypertrophic response to vitamin D_2 affected not only the smooth muscle cells, but also the afibrillar cells and the paucifibrillar cells as well. Very large doses, on the other hand produced necrotic degeneration and hypotension.

Grollman, Harrison and Williams (7) produced hypertension in rats with a number of other sterols, such as desoxycorticosterone, diethylstilbestrol and estradiol benzoate.

It is the purpose of this paper to present the results of observations on blood pressure in rats receiving toxic doses of vitamin D_2. After some preliminary consideration of methods, that of Byrom and Wilson (8) was selected as most suitable with some minor modifications, as follows:

The rat was anesthetized lightly in a battery jar, the top of which was fitted with an ether-soaked cotton pad. The sedated rat was placed on a platform and the tail inserted through a specially constructed cuff modified from that used by Williams, Harrison and Grollman (9). A glass tube filled with water served as a plethysmograph into which the distal extension of the tail was inserted. The proximal end of the plethysmograph was sealed around the tail with vaseline.

The distal end of the plethysmograph was connected with a capillary tube. The meniscus of the water column was adjusted in the capillary tube by a screw plunger in a side arm of the plethysmograph.

An ordinary clinical mercury manometer was attached to the compression cuff but with a T-tube inserted for attachment of the pressure bulb. With the tube clamped between this and the cuff the pressure was raised to 200 mm. Hg. The clamp was then released and the entire pressure suddenly applied so as to shut off

[1] A part of the expenses of this investigation was borne by a grant from the Nutrition Research Laboratories.

[2] Submitted in partial fulfillment of requirements for the degree of Master of Science, 1941.

all arterial flow into the peripheral tail. The pressure was then lowered gradually with the bulb release valve. When the systolic pressure was reached in the compression cuff, there was immediate filling of the tail vessels. The consequent increase in volume caused a sharp movement of the water meniscus in the capillary tube. The manometer was read at this point. Several readings could be made on the same animal before recovery from anesthesia.

EXPERIMENTAL PROCEDURE. Albino rats of both sexes, selected from our own homogeneous colony, were used, with litter mates as controls wherever possible. All of the animals were maintained on a diet of Fox Chow pellets (a commercial preparation) and of tap water ad libitum. They also received weekly rations of lettuce, carrots and whole milk. The experimental rats were fed activated ergosterol[3] dissolved in oil. This was administered by stomach tube, to which the animals very quickly became accustomed. The control rats were fed duplicate volumes of corn oil. Each animal was weighed every 2 to 3 days, and the blood pressure was taken every 3 to 5 days by the technique described. Blood pressure determinations at shorter intervals were deemed inadvisable because of the possible adverse effects of too-frequent ether anesthesia.

Group I comprised twenty rats. After prliminary training and standardization of blood pressure, each animal received a daily dose of 300 units of vitamin D_2 per gram of body weight over a period of 4 days. Toxicity, as manifested by weight loss (upper graphs, fig. 1), was so pronounced that a recovery period was allowed, after which administration was resumed at a daily dose of 30 units per gram as shown in figure 1.

Since this dosage appeared to be innocuous the daily dose was increased to 150 units per gram for 5 days, when weight loss again indicated toxicity. The records of 5 animals, selected at random, were graphed for figure 1. From February 28 to June 15, no observations were made. During the next two weeks a total of 53 readings was made in the group with a mean value of 105 mm. Hg, as compared to a mean of 110 mm. when the last previous reading was made.

With the exception of no. 8, none of the animals in this group showed any significant increase in blood pressure, the mean maximum being 137 mm. Hg at the termination of vitamin D_2 feeding. From figure 1, it will be seen that this hypertension developed during a period of toxication with pronounced weight loss. In *all* other instances weight loss was accompanied by hypotension. Three months later the blood pressure was *98 mm. Hg.* It seems probable, therefore, that this was a true hypertension.

Control group. Eleven litter mate controls were observed for nearly 8 weeks then given volumes of corn oil identical with those in which the vitamin D_2 was given. There was a tendency to progressive increase in the blood pressure almost directly parallel with the increase in body weight. With corn oil feeding for 4 weeks there was a slight tendency to decreased pressure, but this was not marked. After three months the mean of 61 readings was 106 mm., slightly lower than the mean maximum of 117. Due to hot weather the mean weight for the group had decreased also.

[3] Supplied by Abbott Laboratories and Winthrop Chemical Company.

Group II comprised 4 rats (fig. 2). These animals received daily 150 units of vitamin D_2 per gram for 5 days. With prompt weight loss, severe hypotension developed in three animals. Recovery of normotension began with resumption of weight gain 2 or 3 weeks later. Number 19 did not show a very severe weight loss and the hypotension was less severe than in the others. The mean of 8 readings taken three months later was still only 100, although the mean weight was 414 grams, 90 grams more than the previous maximum.

Group III comprised 11 rats that received, daily, 75 units of vitamin D_2 per gram for 56 days. Another control group of like number received identical volumes of corn oil daily for this period. The mean weight gains were identical. It cannot be said that the slight differences in blood pressure readings are significant, although the vitamin group did show a slightly greater mean maximum.

After three months the mean of 45 readings in the vitamin group was 110 mm. Hg as compared to the last previous reading of 124.

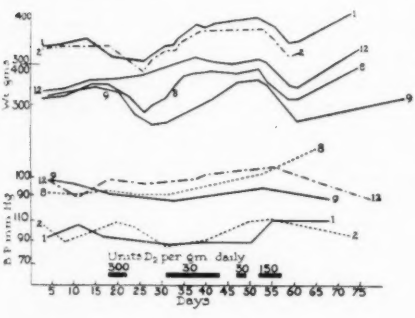

Fig. 1

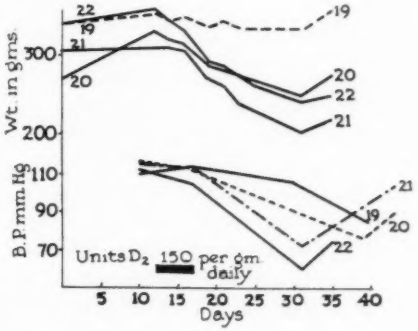

Fig. 2

Fig. 1. Body weight and blood pressure in 5 rats of group 1, receiving vitamin D_2 shown.
Fig. 2. Body weight and blood pressure of 4 rats selected at random from group 2.

Group IV. In order to test the accuracy of the method 6 male rats each received a daily intramuscular injection of 0.5 mgm. of desoxycorticosterone[4] over 10 days. This substance has been proven to be hypertensive for rats, so is regarded as a satisfactory test substance for this purpose. Weight changes during the period were not pronounced, but the mean blood pressure rose from 124 mm. Hg to a maximum of 147 mm. two days after the last injection. Ten days later, hypertension of 135 mm. still persisted. Three months later the mean of 18 readings in this group was 115 mm.

Discussion. The results obtained with desoxycorticosterone prove the method a valid one for studying hypertension in rats. The failure to produce hypertension with daily doses of 150 to 300 units of vitamin D_2 per gram of body weight might be explained as due to the extreme degree of toxicity as manifested by weight loss. However, it might also be expected that such an episode might produce a delayed hypertension. Other studies would lead one to predict this

[4] Supplied by Ciba Company.

result, at any rate, since many of the metabolic disturbances seen in vitamin D_2 toxication persist for 6 to 8 months.

The two groups of observations on corn oil feeding prove that is not a factor of importance in any of the results observed in this study.

The results of administration of vitamin D_2 on the basis of body weight show that daily doses up to 75 units per gram may be tolerated indefinitely without hypertension or weight loss and without any delayed effects. It is in these dosage ranges that one might expect delayed chronic hypertension to develop. This, however, did not occur. In the delayed observations no hypertension was found.

Somewhere between 75 and 150 units per gram (75,000–150,000 per kilo) lies the toxic threshold, where weight loss and hypotension are found. The rat is, therefore, about 5 to 6 times as resistant to vitamin D_2 toxication as the dog and human. According to the recent report of McChesney and Kocher (10), doses of about one-fifth this magnitude are capable of producing hypercalcemia in rats. It appears, therefore, that the hypercalcemic and toxic effects are not closely correlated.

The hypertension that has been reported as occurring in dogs has been explained variously. One explanation is that the vitamin produces arteriosclerosis in the renal arteries, and that the ischemia resulting brings on chronic renin hypertension. Another is that the vitamin produces hypertrophy of the afibrillar or paucifibrillar cells in the preglomerular arterioles. These cells have been charged with responsibility for an endocrine function, i.e., production of a vasopressor substance which, if present in sufficient concentration, would produce vascular spasm and chronic renal ischemia.

Since there have been reports on differences in toxicity among different forms of vitamin D, it now seemed advisable to determine whether other preparations might be more effective in producing hypertension.

The following experiments were designed to test two other products, vitamin D_3[5] and Ertron.[6]

Two groups of albino rats, of the same stock as those used in the earlier work, were used. Each set consisted of two sub-groups, male and female. Each rat served as its own control for a period of 30 to 40 days before the vitamin was introduced.

Group V, comprising 5 females and 4 males, during a control period ranged between 90 and 115 mm. Hg in blood pressure and maintained a steady weight. Vitamin D_3 then was given in a daily dose of 75 units per gram of body weight until death resulted from toxicity. The mean values for the blood pressures and weights for both groups showed a parallel decline. The average values for the males declined from 92 mm. Hg and 308 grams weight to 70 mm. Hg and 235 grams. The final rise (fig. 2) reflects the influence of a last surviving rat. The mean values for the females declined from 95 and 220 to 87 mm. Hg and 151 grams. In no case was hypertension seen.

[5] Courtesy of Dr. J. Waddell of Du Pont Co.

[6] Courtesy of Nutrition Research Laboratories.

Group VI, 4 females and 6 males, averaged between 90 and 120 mm. Hg pressure and maintained a steady weight during the control period. Ertron then was given in a daily dose of 75 units per gram of body weight until death resulted from toxicity. The male group showed a decrease in blood pressure and weight. The female group showed a decline in weight, but there was no significant change in blood pressure. The rise at the end of the experimental period (fig. 3) again was due to a single survivor. In no case was hypertension observed. The results obtained with these products show that neither causes hypertension in rats.

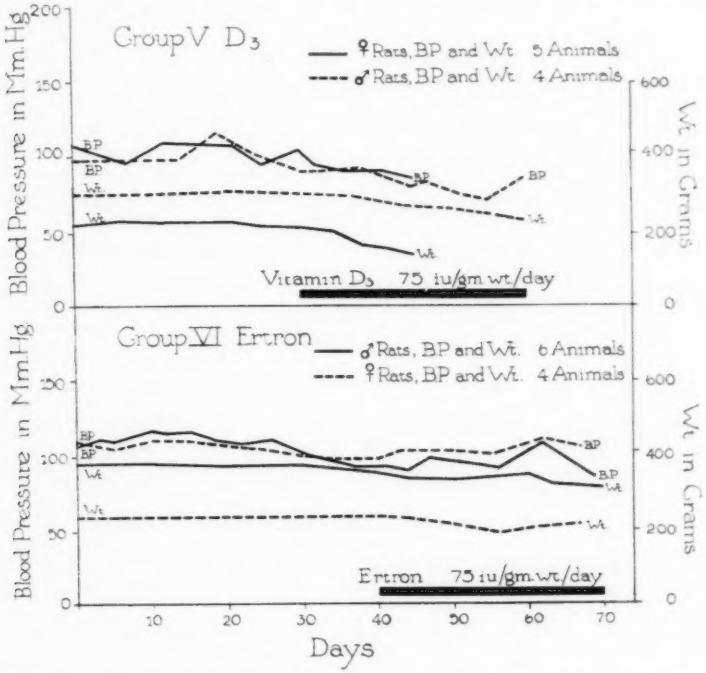
Group V D3
♀ Rats, BP and Wt 5 Animals
♂ Rats, BP and Wt 4 Animals
Vitamin D3 75 iu/gm wt/day
Group VI Ertron
♂ Rats, BP and Wt. 6 Animals
♀ Rats, BP and Wt. 4 Animals
Ertron 75 iu/gm wt/day
Blood Pressure in Mm Hg
Wt. in Grams
Days

Fig. 3. Mean weight and blood pressure of rats receiving vitamin D_3 and ertron. Groups 5 and 6.

These experiments on rats do not confirm the findings of hypertension in dogs after heavy administration of vitamin D_2 reported by others. The reason for the discrepancy is not apparent at present. Frequent attempts have been made in this laboratory to demonstrate in both dogs and humans the production of hypertension by means of various forms of vitamin D, but without success. Further efforts are being made to induce hypertension in dogs.

CONCLUSIONS

1. Blood pressure measured in the tail of the albino rat is not increased, either acutely or chronically, by the oral administration of vitamin D_2 in doses sufficiently large to induce severe toxication as manifested by a marked loss of weight.

2. In growing, untreated, control animals, there is a small increase in blood pressure that is approximately parallel with the weight gain.

3. With loss of weight from toxication the blood pressure tends to be decreased concomitantly with the weight loss.

4. Desoxycorticosterone produces marked hypertension, as reported by others.

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